Stefano Guandalini
Anil Dhawan
Editors

Textbook of Pediatric Gastroenterology, Hepatology and Nutrition

A Comprehensive Guide to Practice

Second Edition

Springer

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Preface to the Second Edition

We are delighted to present the second edition of the *Textbook of Pediatric Gastroenterology, Hepatology and Nutrition* to you all. We were overwhelmed by the interest shown by the readers in the first edition, reflected in many thousand downloads of the chapters.

In the meantime, during the past 5 years or so, a huge amount of research has been published in the field of gastroenterology, hepatology, and nutrition, resulting in a new understanding of the pathophysiological mechanisms of childhood gastrointestinal and liver disorders that has helped to develop newer diagnostics, therapies, and guidelines. It was time to compile the new relevant information in an updated second edition.

Thus, our authors and the editors have worked hard to put together the most useful and up-to-date practical information and present it in the revised chapters.

Furthermore, new authors have been included who are the opinion leaders in their subjects, maintaining and enhancing the international inclusive approach that uniquely keeps characterizing our book.

This edition, like the first one, maintains the practical and ready reference approach for all our readers: trainees, allied healthcare professionals, and established senior practitioners in the field of pediatric gastroenterology, hepatology, nutrition, and transplantation, maintaining the very vision that the late Dr. Branski originally had and that inspired us.

We are humbly confident that we have succeeded in delivering that in this edition as well.

Lastly, we would like to thank the publisher for the enthusiasm and trust in our leadership to help deliver the second edition despite the disruption caused by the Covid-19 pandemic.

We, as editors, are grateful to all the authors for their work and their trust in our role. All of them have carefully and thoroughly reviewed and updated the information by including latest published evidence to enrich the learning experience of the readers and allow them to deliver the best care to all our patients, from babies to young adults.

Chicago, IL, USA — Stefano Guandalini
London, UK — Anil Dhawan

Acknowledgments

I take this opportunity to thank my colleagues who contributed to the second edition of this book for their attention to detail and punctuality.

I would like to thank my wife Anita, who has been behind everything that I have done well in life, and our two boys, Atin and Ashish, for their love and support.

– Anil Dhawan

It's hard to believe 5 years have already gone by since the first edition of this textbook appeared. Besides the original inspiration by the late David Branski, without whose input this book would have never seen the light, I want to acknowledge here not only again all those whom I thanked for the first edition, from my illustrious mentors to the colleagues in ESPGHAN and NASPGHAN and the broader world of my beloved creature FISPGHAN, but also my partners and friends – new and old – at the University of Chicago, who praised this enterprise.

Last but not least, I am sincerely thankful to the unwavering love and support from my wife Greta both during the long years of work and now on my retirement, even more demanding!

– Stefano Guandalini

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Part II Hepatology

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Part I

GI-Nutrition

Microvillus Inclusion Disease and Tufting Enteropathy

1

Agostino Nocerino and Stefano Guandalini

Introduction

The Larger Group of "Intractable Diarrheas of Infancy"

Before focusing on microvillus inclusion disease and tufting enteropathy, we will briefly review similar cases in the literature. In 1968, Avery, Villavicencio, and Lilly were the first to describe severe chronic diarrhea in 20 infants and named it "infantile intractable diarrhea"; according to their description, this was prolonged and intractable despite extensive hospital therapy [1].

This syndrome was defined on the basis of some clinical characteristics, namely: (1) Diarrhea of more than 2 weeks duration; (2) Age, less than 3 months; (3) Three or more stool cultures negative for bacterial pathogens; (4) Necessity of intravenous rehydration; and (5) Prolonged and intractable diarrhea despite hospital therapy.

The death rate was very high: 9 out of the 20 babies (45%) in Avery et al.'s report had died, and at 70% it was even higher in Hyman et al [2].

Heterogeneity and lack of specificity are evident in Avery's original report: different pathologies were grouped in it, some of which with a diagnosis which was well defined even at that time. Only autopsy data were available for the first cases, and only after the introduction of total parenteral nutrition at the beginning of the 1970s [3] was it possible to study the matter in greater depth, thanks to proximal small intestinal biopsy [4] and later on to the development of endoscopic techniques which were safe and adequate for the infant as well. It became consequently possible to discriminate different causes for the so-called intractable diarrhea of infancy [5], but its definition superimposes on the definition of "protracted diarrhea of infancy": the latter lasts for a similar length of time but a failure to gain weight is enough to define the clinical picture [6].

In 1995 the Pediatric Gastroenterologists of the Federico II School of Medicine of Naples (Italy) observed that in most cases of severe and protracted diarrhea (SPD) an etiological diagnosis was possible and that consequently the term "intractable childhood diarrhea" was now frequently inappropriate. They proposed to limit it to the group that needed total parenteral feeding, defining the clinical picture as "severe diarrhea requiring parenteral nutrition" [7]. In view of the changes in the spectrum of known causes of SPD over the past few decades, the Italian Society of Pediatric Gastroenterology, Hepatology and Nutrition (SIGENP) proposed in 1999 [8] to include in this definition autoimmune enteropathy (severe or partial villus atrophy with crypt hyperplasia and presence of anti-enterocyte antibodies and/or associated autoimmune disorders), congenital microvillus atrophy, tufting enteropathy, epithelial dysplasia, and intestinal microvillus dystrophy (the latter later unified with microvillus inclusion disease).

However, the definition of "protracted diarrhea of infancy" has remained prevalent in clinical practice and in the literature, even compared to the broader definition of "pediatric intestinal failure" [9], an entity resulting from various causes including trichohepatoenteric syndrome, tufting enteropathy, microvillus inclusion disease, and autoimmune enteropathy [10].

Many cases of "protracted diarrhea of infancy" are diet-associated, as a consequence of cow's milk or lactose intolerance or malnutrition. Malnutrition causes intestinal atrophy and consequently a malabsorption syndrome with diarrhea, apparently improving with fasting. These features have almost disappeared in developed countries.

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Table 1.1 Main causes of protracted diarrhea in infancy

Small intestinal enteropathy of unknown origin
Intractable ulcerating enterocolitis of infancy
Congenital enterocyte heparan sulfate deficiency
Congenital intestinal integrin deficiency
Congenital secretory diarrheas
Congenital chloridorrhea
Congenital Na-losing diarrhea
Autoimmune enteropathy
Diseases of the intestinal epithelium
Microvillus inclusion disease
Tufting enteropathy

The main causes of "intractable diarrhea of infancy," including more severe and longer-lasting forms, can be summed up as follows (Table 1.1):

Autoimmune Enteropathy

The term "autoimmune enteropathy" (AIE) was introduced to describe persistent diarrhea associated with autoimmune diseases with the production of antibodies directed against epithelial cells of the small and large intestine.

This rare disorder (a recent review of the literature found a total of 98 reports published in the form of case reports and case series) [11] is frequently associated with primary immunodeficiencies and mostly occurs in young infants and children (6–18 months old). It is characterized by severe diarrhea and small intestinal mucosal atrophy resulting from immune-mediated injury. A retrospective study on clinical and histological findings from 40 AIE patients showed a prevalent celiac disease pattern (50%), mainly in patients with primary immunodeficiencies, followed by the mixed pattern (35%), chronic active duodenitis (10%), and GVHD-like pattern (5%) [12]. It remains a challenging diagnosis because of its clinical-pathological variability. This entity is dealt with in Chap. 2.

Small Intestinal Enteropathy of Unknown Origin

This entity could be a variation of autoimmune enteropathy, as the increase in inflammatory cells in the lamina propria shows. It appears in infants less than 12 months, with a lower death rate compared to those with autoimmune enteropathy, but it can be very severe. Infants can become TPN-dependent [5].

Intractable Ulcerating Enterocolitis of Infancy

A rare disease initially described in 1991 in five children presenting in the first year of life with intractable diarrhea, ulcerating stomatitis, and large ulcers with overhanging edges throughout the colon within the first year of life [13]. The affected infants can show a colitis whose severity may require a subtotal colectomy, even if the long-term prognosis is good. It has been suggested that affected children have a genetically determined primary immune dysregulation [14].

Congenital Enterocyte Heparan Sulfate Deficiency

Described in 1995 in three infants who within the first weeks of life presented with secretory diarrhea and massive enteric protein loss [15]. The small intestinal mucosa is normal on light microscopy, but histochemical exams show a complete absence of enterocyte heparan sulfate. The sulfated glycosaminoglycans of the basocellular membrane are mostly deficient, particularly heparan sulfate, while the distribution of vascular and lamina propria glycosaminoglycans is normal [15]. Diarrhea is so severe as to make total parenteral nutrition (TPN) necessary, together with repeated albumin infusions because of severe protein-losing enteropathy. Studies in men and mice show that heparan sulfate is essential in maintaining intestinal epithelial barrier function [16], and that the specific loss of heparan sulfate proteoglycans from the basolateral surface of intestinal epithelial cells is common to many forms of protein-losing enteropathy [17].

Congenital Intestinal Integrin Deficiency

In 1999, Lachaux et al. described a case of intractable diarrhea starting 9 days after birth, associated with pyloric atresia and total epithelial detachment of gastric and intestinal mucosa. Immunofluorescence analysis showed α6β4 integrin deficiency at the intestinal epithelium–lamina propria junction [18].

Mutations in α6 or β4 integrins cause junctional epidermolysis bullosa with pyloric atresia. In 2008, two Kuwaiti brothers with pyloric atresia were described, respectively affected by intractable diarrhea and episodes of protein-losing enteropathy, with a novel mutation in β4 integrin that induced a desquamative enteropathy in infancy without significant skin disease [19].

Congenital Secretory Diarrheas

Includes congenital chloridorrhea and congenital sodium diarrhea, dealt with in Chap. 36.

Diseases of the Intestinal Epithelium

Microvillus inclusion disease and tufting enteropathy are the best-known diseases of the intestinal epithelium causing intractable diarrhea of infancy.

In 1994, Girault et al. described eight infants with early-onset severe watery diarrhea associated to facial deformities and unusual tufts of woolly hair with trichorrhexis nodosa. Duodenal biopsies showed moderate to severe villus atrophy, with normal or hypoplastic crypts; colon biopsies were basically normal. As a consequence, severe malabsorption was present. All patients had no antibody response to immunization antigens; the immunological response to vaccinations was poor. Five children died despite TPN [20]. Two children from the series of Girault et al. had hepatic cirrhosis; six additional patients had signs and symptoms compatible with this new "syndromic diarrhea," associated to hepatic involvement (Trichohepatoenteric syndrome, THES) characterized by fibrotic livers with marked hemosiderosis [21–23].

Nine different mutations in *TTC37* gene (5q14.3–5q21.2) were found in 12 children from 11 families with classical features of THES. *TTC37 codes for a protein that has been named* "thespin" (THES ProteIN) [24].

Enlarged platelets with abnormal α-granule secretion can be observed in some patients. The estimated incidence of the syndrome is 1 in 400,000 to 1 in 500,000 live births.

A review of the literature conducted in May 2017 included 80 patients, 40 with mutations of TTC37 and 14 with mutations of SKIV2. This showed that parenteral nutrition was used in the management of 83% of the patients and that it was possible to wean 44% off parenteral nutrition. The mean duration was 14.97 months. Data on the efficacy of immunoglobulins were reported for only six patients, with a diminution of infection or reduced diarrhea. Antibiotics, steroids, and immunosuppressant drugs were used with little efficacy. Hematopoietic stem cell transplantation (HSCT) was performed in four patients, two of whom died [25].

Microvillus Inclusion Disease

In 1978, Davidson et al. described five infants presenting with intractable diarrhea of infancy characterized by secretive diarrhea and malabsorption, starting in the first hours after birth with hypoplastic villus atrophy in the small intestinal biopsy. Four of these infants had a deceased brother who had shown similar symptoms.

In one of these infants, electron microscopy identified the presence of a peculiar abnormality of the microvilli of the enterocytes [26] (Fig. 1.1).

Three new cases with the same clinical and histological characteristics as this infant were described in France in 1982, and the four of them were grouped into a new disease called congenital microvillus atrophy [27, 28]. Two new cases were described in Great Britain in 1985 [29], and one in Italy in 1986; a brother of the Italian child, who was born subsequently, was similarly affected [30]. A survey completed in 1987 among centers known for their involvement in pediatric gastroenterology identified more than 30 cases worldwide. Additional cases were later published.

In 1989, Cutz et al. proposed the use of the term "microvillus inclusion disease" to highlight the characteristic ultrastructural lesions of the disease [31].

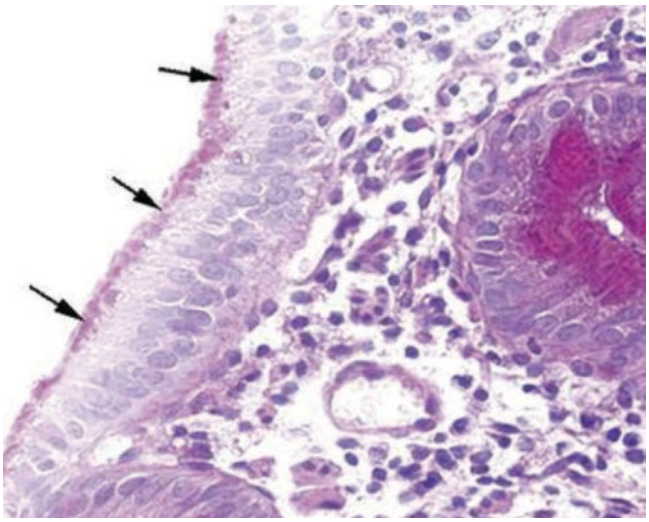

Fig. 1.1 Microvillus inclusion disease. PAS staining highlights abundant PAS-positive material (*arrows*) in the apical part of the enterocyte cytoplasm. PAS × 260 [20] (Reprinted from Springer and Virchows Archive: Official Journal of the European Society of Pathology, Morroni et al. [99], Fig. 1, with kind permission from Springer Science and Business Media)

Clinical Presentation

First child of parents with no blood relation, A.G. was born after 37 weeks of gestation, the pregnancy having been complicated by a risk of miscarriage in the fifth month. His weight at birth was 3500 grams.

The infant was hospitalized when he was 40 days old because of abundant diarrhea (15–20 evacuations a day of liquid stools), which started on the sixth day of life and was resistant to numerous dietary and pharmacological therapies.

On admission to hospital, the patient weighed 2800 grams, and was suffering from dystrophia and dehydration; total parenteral nutrition (TPN) was therefore immediately started. The acid-basic balance showed hyponatremic acidosis (pH 7,2; EB –8,3; Na 128 mEq/1). The secretive nature of diarrhea was confirmed by its entity (about 100 ml/kg/die) with a total absence of oral nutrition and with the persistence of TPN in progress.

Moreover, the typical absence of ionic gap in the stools was present: osmolality 226 mOsm/l, Na 86 mEq/1, K 23.5 mEq/1 (gap 7 mOsm/l).

Loperamide and chlorpromazine increased intestinal absorption but did not change the clinical picture.

Microbiological tests including electron microscopic analysis of the feces for the identification of viruses and the search for enterotoxigenic bacteria and parasites with specific methods were repeatedly negative.

The abdominal ultrasound showed adrenal hyperplasia associated with hyperaldosteronism (1160 ng/ml, v. n. <125 ng/ml).

Jejunal biopsy showed a picture of villus atrophy with no hyperplastic crypts and periodic acid-Schiff (PAS)-positive material stored in the apical cytoplasm of enterocytes. Electron microscopy was diagnostic for microvillus inclusion disease.

Microvillus Inclusion Disease: A Congenital Secretory Diarrhea Starting in Neonatal Age

In most cases, severe diarrhea appears in the first days of life, usually within the first 72 h, and is immediately life threatening. The stools are watery, and the stool output is 100–500 mL/kg/d when the infant is fed, a volume comparable to or higher than that observed in cholera. The diarrhea is of secretory type; therefore, it persists at a stable rate of 50–300 mL/kg/day despite fasting, and the electrolyte content of the stools is increased, without an osmotic gap. However, the mucosal atrophy causes additionally osmotic diarrhea in presence of luminal nutrients. For this reason, feeding increases the fecal output, and oral feeding in nutritionally significant amounts is impossible. Due to the high output, patients can lose up to 30% of their body weight within 24 h, resulting in profound metabolic acidosis and severe dehydration, unless vigorous intravenous rehydration is started.

Microvillus inclusion disease is the leading cause of secretory diarrhea in neonates with onset in most cases within the first few hours after birth [32]. However, in a small percentage of cases (currently considered around 5% of total) [33], diarrhea starts later in life: between 1 and 3 months, and more commonly at 6–8 weeks of age. This less severe form has been denominated late-onset microvillus inclusion disease, while the classical form beginning at neonatal age has been denominated early-onset microvillus inclusion disease [34].

A few cases have been termed atypical microvillus inclusion disease, in which the onset can be early or late, but the histological picture is different, particularly for the absence of detectable microvillus inclusions. The first case was a 5-month-old Navajo with profuse diarrhea beginning on the sixth day of life, who did not have microvillus inclusions in the duodenal tissue; a second biopsy confirmed the absence of classic microvillus inclusions despite the lack of surface microvilli and the presence of cytoplasmic vesicular bodies. However, a few microvilli associated with cytoplasmic inclusions were observed in a third biopsy [35]. In consideration of the typical clinical presentation, a diagnosis of microvillus inclusion disease was made, instead of intestinal microvillus dystrophy proposed for other cases with similar ultrastructural findings but slightly atypical clinical presentation [36]. Other similar cases were observed later.

Therefore, three variants of the disease have been identified: early-onset microvillus inclusion disease, late-onset microvillus inclusion disease, and atypical microvillus inclusion disease.

1. Early-onset MVID presents a complete loss of intestinal proteins, both in the villi and in the crypts.
2. In the late-onset MVID, there are normal microvilli at the base of the villi and in the crypts. The clinical picture is less severe and occurs later, usually from the second month of life.
3. In the atypical MVID, the microvillus proteins are absent or defective only at the crypts, while in the villi there are normal microvilli at the villus surface.

However, because of the sparse distribution of microvillus inclusions, it is not certain that their absence could not be limited to the sample.

The disease is characterized by defective transport of plasma membrane proteins to the apical brush border, due to mutations of the MYO5B gene on chromosome 18q21 [37, 38], encoding myosin Vb motor protein and two small GTP binding proteins, Rab8 and Rab11 [39].

Several mechanisms responsible for the pathological picture of MVID have been suggested, and in particular the presence of defects in vesicle trafficking or delivery (*Trafficking model*), in the recycling and delivery of apical recycling endosomes (*Recycling model*) or in the colocalization of ezrin and ezrin kinase in apical recycling endosomes (AREs), while ezrin kinases are normally transported to the apical membrane where they activate ezrin (*Local induction model*). It is possible that these mechanisms coexist, and so a hybrid model that combines all three models has been proposed [40].

The hallmark of the disease is the electron microscopic finding of disrupted enterocytic microvilli (i.e., digitations of the apical membrane of the intestinal epithelial cell protruding into the lumen) without inflammatory changes and the appearance of characteristic inclusion vacuoles, whose inner surfaces are lined by typical microvilli. Both lesions are seen only with the electronic microscopy.

The main histological features of the disease include diffuse villus atrophy without inflammatory changes and accumulation of periodic acid-Schiff (PAS)-positive material within the apical cytoplasm of enterocytes. The definitive diagnosis of MID rests on distinctive ultrastructural findings: microvillus inclusions (more frequently in villus enterocytes), increased electron-dense secretory granules (preferentially in crypt epithelial cells), and poorly developed brush border microvilli on the intestinal surface epithelium.

Microvillus inclusion disease is usually characterized by growth retardation and some developmental delay later in infancy. While no other specific findings can be detected, the disease can be associated with other abnormalities, indicated in Table 1.2.

Some cases of microvillus inclusion disease associated with other clinical pictures (for example, cardiac malformations, facial dysmorphia, transient neuronal dysplasia, aganglionic megacolon, Down syndrome, intrahepatic cholestasis, and hypochondroplasia) have been described. In a series of 24 patients with MVID followed up from birth to 23.5 years, liver disease was recorded in 22 patients, kidney disease in 9, and pulmonary disease in 2 cases [41].

Table 1.2 Anomalies described in association to microvillus inclusion disease

Meckel diverticula	Abdominal adhesions
Inguinal hernias	Renal dysplasia
Absent corpus callosum	Hydronephrosis
Mesenteric duct remnants	Craniosynostosis
Abnormal vertebrae	Down syndrome
Aganglionic megacolon	Hematuria
Pneumocystis jiroveci pneumonia	Dihydropyrimidinase deficiency
Autosomal dominant hypochondroplasia	Microcephaly
Renal Fanconi syndrome	Other renal problems
Hypophosphatemic rickets	Diabetes
Cardiac problems	Pulmonary problems
Liver dysfunction	Multiple hepatic adenomas

An infant who had presented on the second day of life with the first symptoms of necrotizing enterocolitis was diagnosed with congenital microvillus atrophy at the age of 2.5 weeks. The clinical picture of necrotizing enterocolitis was repeated at the age of 4, 7, and 11 weeks of life and was treated with antibiotics on a monthly basis. The authors suggested that the picture of necrotizing enterocolitis was caused by damage to the barrier function of intestinal epithelial cells [42].

A series of eight children aged between 2 days and 14 months at onset, six of whom were homozygotes or compound heterozygotes for MYO5B mutations, were observed with minor microscopy histological abnormalities, sometimes focal or delayed but consistent with MVID. Malformations and severe mental retardation were observed in three cases, and hydrocephaly in one [43].

An infant with microvillus atrophy presented with liver dysfunction, hematuria, and *Pneumocystis jiroveci* pneumonia during the course of the disease; the child succumbed after massive pulmonary hemorrhage. The authors hypothesized that the coinfections could have been facilitated by an altered MYO5B function [44].

MVID-associated liver dysfunction similar to progressive familial and benign recurrent intrahepatic cholestasis has been described in other cases, to suggest that MVID is not exclusive to the intestine.

Histologic Findings

Findings from duodenal biopsy must not be considered diagnostic. Histologic results of duodenal biopsy samples can range from essentially normal to mildly abnormal, showing the following:

- Thin mucosa caused by hypoplastic villus atrophy
- Diffuse villus atrophy (loss of villus height)
- Crypt hypoplasia

Periodic acid-Schiff (PAS) staining of the intestinal biopsy sample does not show the usual linear staining along the brush border but reveals PAS-positive material in the apical cytoplasm. The PAS staining material corresponds to the increased number of electron-dense secretory granules in the epithelium. The abnormal pattern of staining appears in the upper crypt region and continues over the villus [45] (Fig. 1.2).

PAS accumulates in low crypts in atypical microvillus atrophy, in upper crypts in congenital microvillus atrophy, and in low villi in late-onset microvillus atrophy.

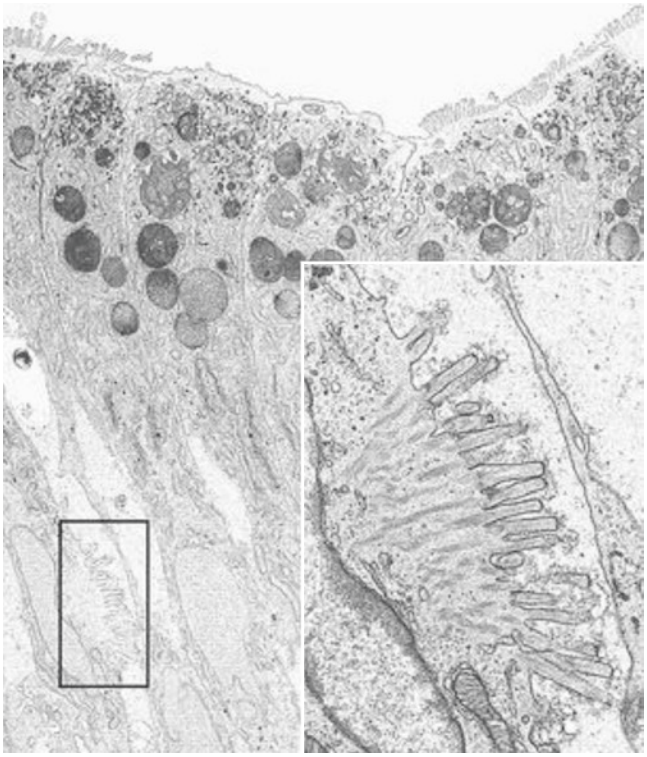

Fig. 1.2 Microvillus inclusion disease. Villus enterocytes: the *boxed area* shows microvilli on the lateral membrane. *Inset*: Enlargement of the boxed area. ×6200, *inset* ×22,500 [20] (Reprinted from Springer and Virchows Archive: Official Journal of the European Society of Pathology, Morroni et al. [99], Fig. 5, with kind permission from Springer Science and Business Media)

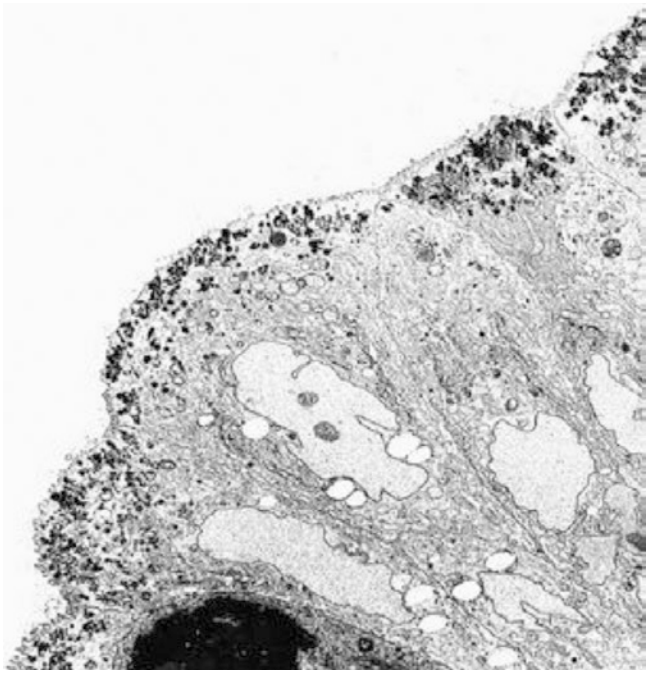

Fig. 1.3 Microvillus inclusion disease. The apical cytoplasm of villus epithelium shows an increased number of secretory granules associated with microvillus alterations. ×2400 [20] (Reprinted from Springer and Virchows Archive: Official Journal of the European Society of Pathology, Morroni et al. [99], Fig. 4, with kind permission from Springer Science and Business Media)

Similar results were obtained with anti-CD10 immunohistochemistry: in affected children the normal linear staining in surface enterocytes is absent, while prominent cytoplasmic reactivity is seen. CD10 is a neutral membrane-associated peptidase; thus, abnormal stain findings with PAS or anti-CD10 immunohistochemistry are expressions of the abnormalities in microvillar structure.

Rectal biopsy findings demonstrate microvillus involutions and an increased number of secretory granules. This test has been proposed as a relatively easy method for making an early diagnosis. Anti-CD10 immunohistochemistry can aid in the diagnosis, because abnormal cytoplasmic CD10 staining of absorptive colonocytes has been observed in microvillus inclusion disease [46], although while CD10 immunostaining identifies normal enteric mucosa with 100% specificity, negative staining does not definitively exclude small intestinal mucosa in the setting of active enteritis, a common condition in ileal pouch mucosa.

The diagnosis rests on findings demonstrated by application of immunohistochemical stains for microvilli antigens, such as villin or CD10, and electron microscopy (EM) [47] (see Figs. 1.3 and 1.4).

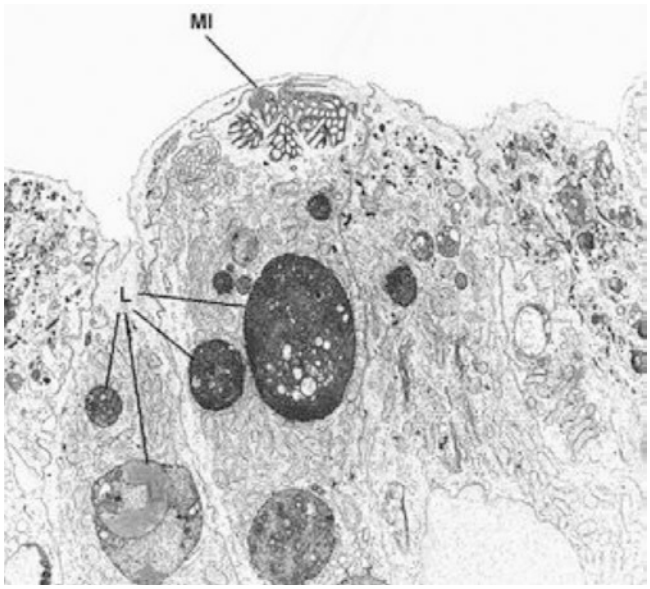

Fig. 1.4 Microvillus inclusion disease. The villus enterocytes lack brush border microvilli, whereas their apical cytoplasm contains a microvillus inclusion (MI) and numerous lysosomes (L) ×5.500 [20] (Reprinted from Springer and Virchows Archive: Official Journal of the European Society of Pathology, Morroni et al. [99], Fig. 2, with kind permission from Springer Science and Business Media)

Electron microscopy shows well-preserved crypt epithelium with abundant microvilli. Villus enterocytes are severely abnormal, particularly toward the apices of the short villi. The microvilli are depleted in number, short, and irregularly arranged. Some of the enterocytes contain the typical microvillus involutions, which are intracellular vacuoles where microvilli are observed lining the inner surface. Transmission electron microscopy (TEM) can efficiently demonstrate both the absence of surface microvilli, microvillus disorganization, and intracytoplasmic microvillus inclusions in enterocyte cytoplasm containing cryptic microvilli [48].

A striking feature is the finding of several small, membrane-bound vesicles containing electron-dense material (see Figs. 1.3 and 1.4). A few cases have been described in which the classic microvillus inclusions are shadowed by other features, such as large aggregates of electron lucent, vermiform membranous vesicles in enterocyte cytoplasm, corresponding to the PAS-positive material [49].

Epidemiology

Congenital microvillus atrophy is a rare disease. By 2014 only 137 cases had been published or gathered in an online registry [33]; to date around 200 cases of MVID have been reported, with a prevalence <1/1000.000, apparently more numerous in countries where marriages between blood relatives are more frequent [33].

A female preponderance had been observed among the published cases, with a female-to-male ratio of 2:1, but in the total reported 137 cases, there is instead a 1.54 male/female ratio. Consanguinity is present in 41% of the assessable cases with a gender preference for males. A cluster of cases from the Navajo reservation in northern Arizona suggests an incidence as high as 1 case per 12,000 live births [50].

Pathophysiology

Due to their alterations, mature enterocytes inefficiently absorb ions and nutrients, causing a malabsorption syndrome; however, the diarrhea is caused mainly by active secretion of water and electrolytes in the intestinal lumen (secretory diarrhea). The pathogenesis of the secretory diarrhea is unknown; it is believed to result from an unbalance between decreased absorption and unaltered secretion.

Measurement of stool electrolytes and osmolality enables rapid and accurate assessment of the pathogenesis of this chronic diarrhea (osmolar vs secretory) and greatly narrows the differential diagnosis.

Fecal electrolytes demonstrate a typical pattern of secretory diarrhea. Fecal sodium levels are high (approximately 60–120 mEq/L), and no osmotic gap is found. In patients with secretory diarrhea, the following formula applies: 2 × (Na concentration + K concentration) = stool osmolarity± 50. In osmotic diarrhea, stool osmolarity exceeds 2 × (Na concentration + K concentration) by 100 or more.

Secretory diarrhea occurs in the fasting state and is associated with large output losses that cause dehydration and metabolic acidosis.

In osmotic diarrhea, findings on stool microscopy are negative for white blood cells (WBCs), blood (exudative diarrhea), and fat (steatorrhea).

Even if there are data about the anomalies in water and electrolytes transport in the small intestine, it is not known whether and how the colonic mucosa participates in the absorption alterations in the disease.

In one of the Italian cases, we used the technique of rectal perfusion that showed a decrease in sodium absorption, only partially corrected by chlorpromazine administration [51].

Pathogenesis

Severe perturbation of the microvillar cytoskeleton may disrupt the transport of brush border components that have to be assembled at the apical membrane. The postulated abnormality in the cytoskeleton causes a block in exocytosis, mainly of periodic acid-Schiff (PAS)-positive material (e.g., polysaccharides, glycoproteins, glycolipids, neutral mucopolysaccharides). Consequently, small secretory granules that contain a PAS-positive material accumulate in the apical cytoplasm of epithelial cells.

Genetic evidence of the link between MVID and apical vesicle trafficking was initially obtained in Rab8 knock-out (KO) mice [52].

In 2008, the presence of mutations in the Myosine Vb (MYO5B) gene was described in seven patients (out of ten tested), predominantly of Turkish origin [37]. Homozygous mutations in the same gene were subsequently found in seven cases of Navajo origin; five parents were heterozygote [38]. A total of 41 unique MYO5B mutations had been identified [33]. Most patients with early-onset MVID display inactivating mutations in the MYO5B gene, one of the three myosin-5 genes (MYO5A, MYO5B, MYO5C) present in mammals, which are implicated in the spatiotemporal segregation and the transportation of organelles.

The MYO5B gene codifies Myosine Vb, an actin-based motor protein which carries the recycling endosomes to the apical plasma membrane along the actin filaments of the microtubules after having bound to a specific small guanosine-5'triphosphatase (GTPase) rab protein, such as Rab 11A, Rab8A, and RAB8A, located on the surface of recycling endosomes [53]. Myosine Vb mediates the tethering of Rab guanosine triphosphates, which determines apical vesicle transport and membrane recycling [54].

Studies in neonatal mouse models have shown that loss of active MYO5B causes early diarrhea, failure to thrive, evident microvillus inclusions, and loss of apical transporters in the duodenum. By contrast, induction of MYO5B loss in adult mice led to the rapid onset of diarrhea, but did not induce the formation of significant numbers of microvillus inclusions, to suggest that the formation of microvillus inclusions in duodenal enterocytes is far more pronounced in neonates, in whom we see a loss of proper trafficking and recycling of transporters to the apical brush border in enterocytes [55].

Thanks to this link the recycling endosomes move along the actine filaments [56] (*See* Fig. 1.5). The functional deficiency of Myosine Vb causes impaired microvillus formation and defective epithelial polarization [48]. The MYO5B mutant proteins are unable to bind to either RAB8A or RAB11A, with consequent microvillus structural defects, and the recycling endosomes are not carried in a normal way: in the enterocytes of the subjects with microvillus inclusion disease, no regular accumulation of myosine Vb and of the recycling endosome-associated proteins (one of these is Rab 11) can be observed close to the apical membrane, and no specific staining pattern is present [57]. Consequently, liquids and foods are not absorbed sufficiently, resulting in diarrhea, hypo-nutrition, and dehydration.

Therefore, Rab 11 distribution in the enterocytes can be a helpful diagnostic tool [58]. However, the spectrum of cellular defects in MVID is heterogeneous, and the severity of villus blunting, the ectopic formation of basolateral microvilli, the presence of ultrastructural features of microvillus inclusions (MVIs) may vary significantly. The cellular basis of this variability is still under study. In vitro cell-based mod-

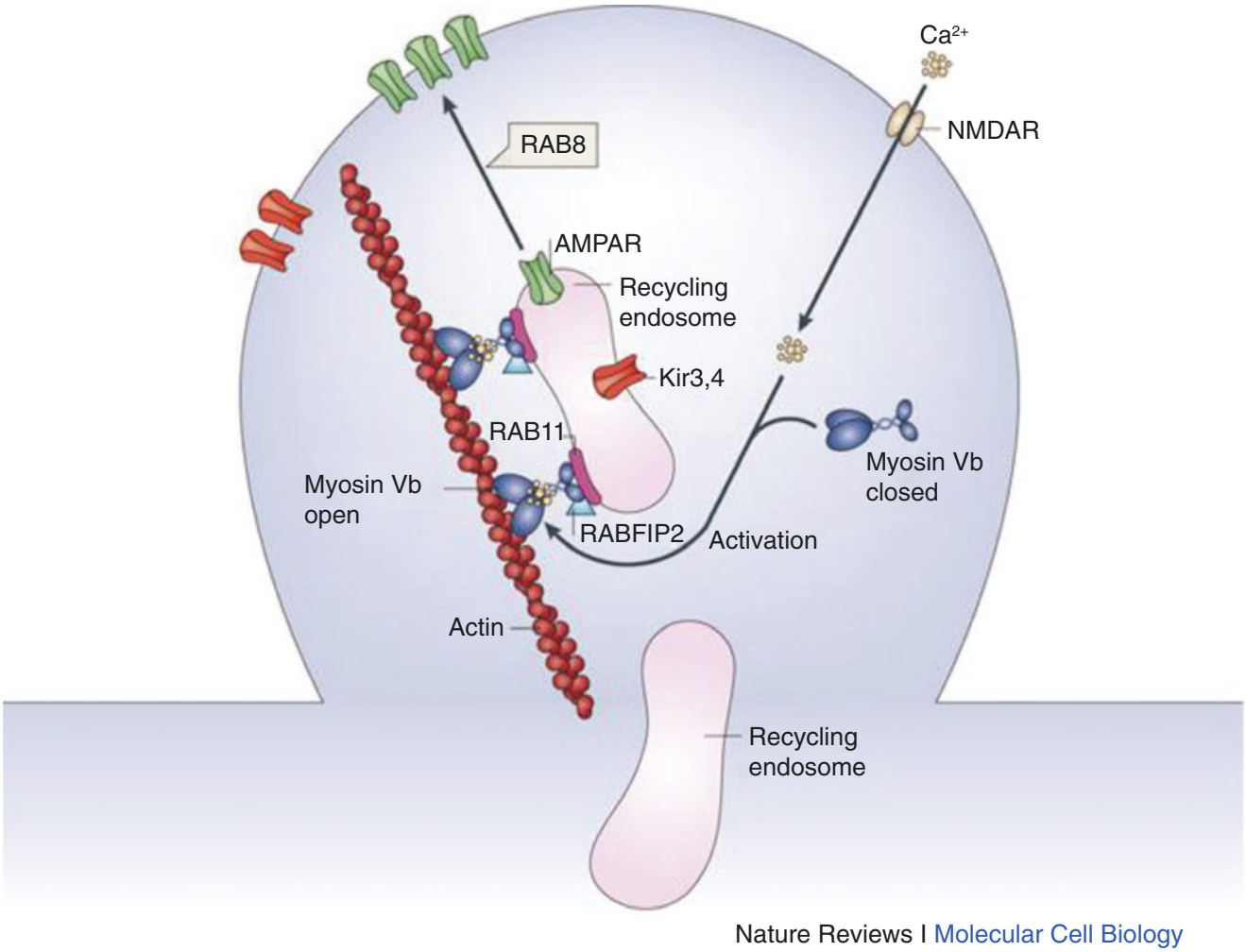

Fig. 1.5 Endocytic recycling. Myosin Vb is a conformation-dependent binding partner of Rab11-FIP2. Activation of myosin Vb induces translocation of recycling endosomes and their cargo. Final transport from the recycling endosome to the cell surface is mediated by Rab8 [37] (Reprinted by permission from Macmillan Publishers Ltd. and Nature Publishing Group: Nature Reviews Molecular Cell Biology, Grant and Donaldson [56])

els proved useful for greater understanding and showed that the MVID phenotype is correlated with the degree of enterocyte differentiation [59].

Molecular analysis strongly contributes to the unequivocal diagnosis of MVID and even prenatal diagnosis. So far about 60 different mutations have been identified, indicating the strong genetic heterogeneity of the disease.

Other biochemical mechanisms depending on myosin Vb which can produce alterations in the structure of the microvilli are presently being studied [60].

Myosin Vb is expressed in all the epithelial tissues and, as a matter of fact, microvillus inclusions in the stomach and colon, in addition to less well-defined inclusions in gallbladder epithelium and in renal tubular epithelial cells, have been reported in some patients with microvillus inclusion disease (MVID). Nevertheless, no extraintestinal symptoms are generally reported. Two children with renal Fanconi syndrome who carried mutation MYO5B did not show alterations in the apical brush border morphology and the PAS staining pattern in renal tubular epithelial cells, which makes it unlikely for it to be the cause of proximal tubular renal dysfunction [61].

At present it is not possible, from molecular analysis, to predict the outcome of the disease, since the prognosis depends mainly on early treatment, including intestinal transplantation.

Recent evidence shows that the effects of mutations in MYO5B are not limited to the small intestine but extend to other organs such as the liver, colon, pancreas, stomach [62], and bile salt export pump (BSEP) in the canalicular membrane, contributing to cholestasis [63].

In 2014, Dutch investigators found that the mild variant of MVID appears to be caused by loss of function of syntaxin 3 (STX3), an apical receptor involved in membrane fusion of apical vesicles in enterocytes [64]. STX3 mutations were identified by whole exome sequencing in two patients diagnosed with MVID based on clinical symptoms but without MYO5B mutations. In fact, whole exome sequencing of DNA from patients with variant MVID revealed homozygous truncating mutations in STX3, and in addition, patient-derived organoid cultures and overexpression of truncated STX3 in CaCo2 cells recapitulated most characteristics of variant MVID.

Mutations in STXBP2, a gene that encodes the syntaxin-binding protein-2 (also called mammalian uncoupled munc18–2 protein) which as STX3 may have a role in membrane fusion, have also been identified in patients with severe chronic diarrhea starting shortly after birth without signs of infection [65], as well as in patients with familial hemophagocytic lymphohistiocytosis type 5 (FHL5, OMIM 613101), a hyperinflammatory immune disorder which in 40% of cases is associated with severe chronic diarrhea starting shortly after birth.

It has been suggested that MYO5B, STX3, and STXBP2 are part of a common disease mechanism that unifies a subset of phenotypically linked congenital diarrheal disorders, regulating protein trafficking to the apical brush border [66].

Prenatal Diagnosis

Pregnancy and birth are usually normal in individuals with microvillus atrophy. However, cases with severe prognosis have been reported [67]. Polyhydramnios has been reported very rarely [33], in contrast to the clinical picture of patients with other causes of congenital secretory diarrhea, and antenatal and natal periods are usually uneventful.

Nevertheless, in some cases, polyhydramnios and bowel dilation in the third trimester have been described. In one case, a high fetal alpha-fetoprotein in the second trimester was observed [68]. Authors have speculated that the fetal alpha-fetoprotein elevation might possibly be caused by in utero body fluid leakage into the amniotic fluid through fetal enteropathy.

Identification of the gene responsible for the disease allows its prenatal diagnosis [69].

Treatment

The prognosis of early-onset microvillus inclusion disease is poor. If patients are untreated, the disease is rapidly fatal because of dehydration and malnutrition.

In late-onset microvillus inclusion disease diarrhea tends to be less severe, and some alimentation is possible.

Medical Care

Agents tentatively given to induce a better growth of the intestinal mucosa (e.g., epithelial growth factor, colostrum) are ineffective. Several drugs (e.g., corticosteroids, cromoglycate disodium, cimetidine, somatostatin, octreotide, loperamide, chlorpromazine, urogastrone/epidermal growth factor) have been tried to counteract the massive secretory diarrhea in patients with microvillus atrophy; however, none has proven effective.

In a 4-year-old boy diagnosed with congenital atrophy of the microvilli, after unsuccessful treatment with loperamide (0.2 mg/kg 4 times a day), racecadotril therapy proved to be effective [70]. The drug reduces the degradation of the enkephalins, abundant in the intestinal villi, and has an antisecretory effect through the inhibition of the cyclic adenosine monophosphate (cAMP) [71].

At present, the only available therapy is total parenteral nutrition (TPN). Children with late-onset microvillus inclusion disease usually have less severe diarrhea; as they get older, TPN can be reduced to once or twice per week.

If patients are treated with TPN, their prognosis entirely depends on the complications of this approach. These complications include cholestasis with subsequent liver damage leading to cirrhosis, catheter-related sepsis due to infection with bacterial or fungal agents, and progressive lack of vascular access.

In the observed cases, cholestasis appears to be worsened by transplantation.

The study of eight patients who developed cholestatic liver disease suggests that cholestasis is enhanced by the impairment of the MYO5B/RAB11A apical recycling endosome pathway in hepatocytes [72].

Surgical Care

Successful outcomes of small intestinal transplantation have been reported, and evidence suggests that an early transplant might be beneficial. The limited experience accumulated in a few centers worldwide reflects an overall survival rate of approximately 50% at 5 years after small-bowel transplantation; this is a much better outcome than is seen with other indications for intestinal transplantation [73]. Patients who did not receive colonic transplant weaned later from parenteral nutrition.

The analysis of 16 patients who underwent a small-bowel transplantation shows a lower death rate compared to those who did not (23% versus 37%) after an average 3.5-year observation period (but variable between 3 months and 14 years). In all the cases, apart from the first two, the colon had been transplanted too [74].

Although only small series have been reported, evidence suggests that early small-bowel transplantation should be performed, at least in children with early-onset microvillus inclusion disease. Patients with late-onset microvillus atrophy appear to have an improved prognosis.

Transplantation appears to be the only option for patients who do not fare well with long-term TPN (e.g., because of sepsis, liver damage, lack of vascular access). For patients in whom transplantation is successful, a gradual return to a normal diet is considered possible.

In the observed cases, TPN-related cholestasis appears to be made worse by transplant. Therefore, in children with cholestasis, the worsening of this picture after the transplant points to a combined liver-intestinal transplantation.

Tufting Enteropathy (or Intestinal Epithelial Dysplasia)

In 1994, Reifen et al. described two infants less than a month old with protracted diarrhea. The diarrhea was so profuse to make total parenteral nutrition (TPN) necessary but it improved when enteral nutrition was interrupted. The jejunal biopsies showed a peculiar picture characterized by the presence of focal aggregations of packed enterocytes in the shape of a teardrop, as a consequence of an apical rounding of the plasma membrane. These focal areas looked like tufts and that is why the term "tufting enteropathy" was coined [75]. Curiously, a case with the same characteristics was identified among those presented by Davidson et al. in the same paper where the first case of microvillus inclusion disease had been described [26].

According to current criteria, diagnosis is based on the presence of total or partial villus atrophy associated with crypt hyperplasia, in the absence of signs of inflammation, associated with the characteristic focal localized epithelial tufts, whose presence is an element of distinction from two other enteropathies that directly affect enterocytes, the microvillus inclusion disease, and the trichohepatoenteric syndrome. The tufts are formed by enterocytes enclosed in a plasma membrane, located in the duodenum and jejunum.

Clinical Expression

The incidence of the disease has been estimated to be rare, with a prevalence of 1:50.000–1:100,000 live births in Western Europe [76], but it seems higher in people of Arab origin.

The vast majority of the mutations in the EpCAM gene that cause the disease have been identified in patients originating from Europe, North West Africa, and the Mediterranean area and from Saudi Arabia in particular. The incidence rate is higher in areas with high proportion of close relatives and in the Arab region than in other regions [77]. However, reports from Asia are very scarce, and the incidence of tufting enteropathy in this area is unknown; the cases published so far include only two patients from South Korea and one from China [78].

The clinical picture is characterized by a severe secretory diarrhea, generally with loose stools, starting in the first weeks of life. During pregnancy, there is no polyhydramnios, as in the microvillus inclusion disease and differently from congenital sodium diarrhea and congenital chloridorrhea.

The alterations in the enterocytes in any case cause an accentuation of the diarrhea with nutrition, including total enteral nutrition, as had already been observed from the very first cases described. The histological analysis of the intestine shows anomalies of the basement membrane, disorganization of the enterocytes, and a "crowding" at the apex of the villi which are arranged like tufts.

There are two different clinical forms: one is isolated and the other is syndromic, associated with various anomalies, particularly facial dysmorphism with choanal atresia and superficial punctuated keratitis [79, 80], together with reduced body size and immunodeficiencies.

However, the clinical picture can be confusing. Three cases of tufting enteropathy associated with chronic arthritis and one diagnosed with juvenile rheumatoid arthritis, treated with prednisolone, have been described [81, 82].

Pathophysiology

The clinical picture is mainly caused by an abnormal development of intestinal epithelial cells, which are destroyed and grouped into clusters.

In 2008, a biallelic mutation of the gene for the epithelial cell adhesion molecule (EpCAM gene) was identified in five affected children, two of whom belonged to the same family [83].

Subsequently, other mutations were identified, the main ones of which are located in exons 3, 4, and 5, and cause a deletion of the extracellular and transmembrane regions of the EpCAM protein [84]. The EpCAM is a Type I superficial glycoprotein that is expressed on the surface of the basolateral membrane of many epithelial cells, with a fundamental role in the structural integrity and adhesion of epithelial tissues [85]. The mutant EpCAM accumulated in the endoplasmic reticulum is co-localized with GRP78/BiP, a reticulum chaperon. It has therefore been hypothesized that a response through a protein pathway may be induced in the endoplasmic reticulum [86].

In 2010, a mutation in the SPINT2 gene was found in a case affected by a syndromic form of tufted enteropathy. SPINT2 is a transmembrane protein which seems to be involved in epithelial regeneration [87], whose mutations may result in an indirect loss of EpCAM protein, due to activation of matriptase, a type II transmembrane serine protease expressed in most human epithelia, which causes its proteolysis [88]. Mutations in SPINT2 (MIM# 605124) have been implicated in a syndromic form of the disease, which may cause an indirect loss of EpCAM protein due to proteolysis by the activation of matriptase [89].

It is interesting to note how mutations in the SPINT 2 gene are also present in the syndromic congenital sodium diarrhea, where choanal atresia, hypertelorism, and corneal erosions are particularly frequent and anal atresia can be found in certain cases [90].

The analysis of 57 patients revealed mutations in the gene for EpCAM in 73% of the cases, all of them presenting with an isolated intestinal disease, but in 21% of cases, all with a syndromic form of the disease, mutations of the SPINT2 gene were present [90].

According to this study, tufting enteropathy could be separated into at least three genetic classes, each with specific phenotypes.

However, it seems impossible at present to distinguish between tufting enteropathy and syndromic enteropathy, even from a genetic point of view.

Histological Features

Jejunal biopsy shows a picture of partial villus atrophy together with crypt hyperplasia. The most characteristic feature, the one which gave the name to the disease, is the presence of "tufts," small focal aggregates of teardrop-shaped enterocytes with apical rounding (*see* Fig. 1.6a, b) [91], in addition to characteristic focal epithelial tufts composed of enterocytes with plasma membrane rounding found in the duodenum and jejunum.

The "tufts" are not a characteristic exclusive to intestinal epithelial dysplasia, because they have been observed in other mucosal enteropathies and in normal jejunum. In the latter cases anyway, they were present in <10% of the epithelial surface, while in "tufting enteropathy," they are present in more than 80% of the jejunal surface. But the picture is not always so evident in the earliest period of the disease. Attempts at immunohistochemical analysis (including beta-catenin, E-cadherin, desmoglein, and laminins) have not been easy applicable [92]. On the contrary, staining with EpCAM/MOC31 antibody, an EpCAM antibody clone, showed a sensitivity and specificity of 100% for loss of staining in 15 studied patients [93].

Electronic microscopy shows relatively normal microvilli, and it may be useful as a diagnostic tool only to exclude microvillus inclusion disease.

Mild inflammation of the lamina propria is also present. Infiltration of T lymphocytes within the lamina propria had always been observed since the original description, even if inferior to celiac disease, but it sometimes gives rise to a suspicion of autoimmune enteropathy [75].

Treatment

Tuft enteropathy is associated with severe secretory diarrhea, which worsens with nutrition. That is why affected children have to be treated with total parenteral nutrition (TPN).

Some cases seem to have a less severe course and they can be given a partial parenteral nutrition [94].

There is currently no specific treatment for tufting enteropathy. Many cases are treated with long-term parenteral nutrition, which it may be possible to reduce or suspend with increasing age [95]. Twelve patients survived for 8–30 years under long-term parenteral nutrition therapy. In those cases where it is impossible to continue parenteral nutrition, or when there were other serious issues, bowel transplantation was attempted [96].

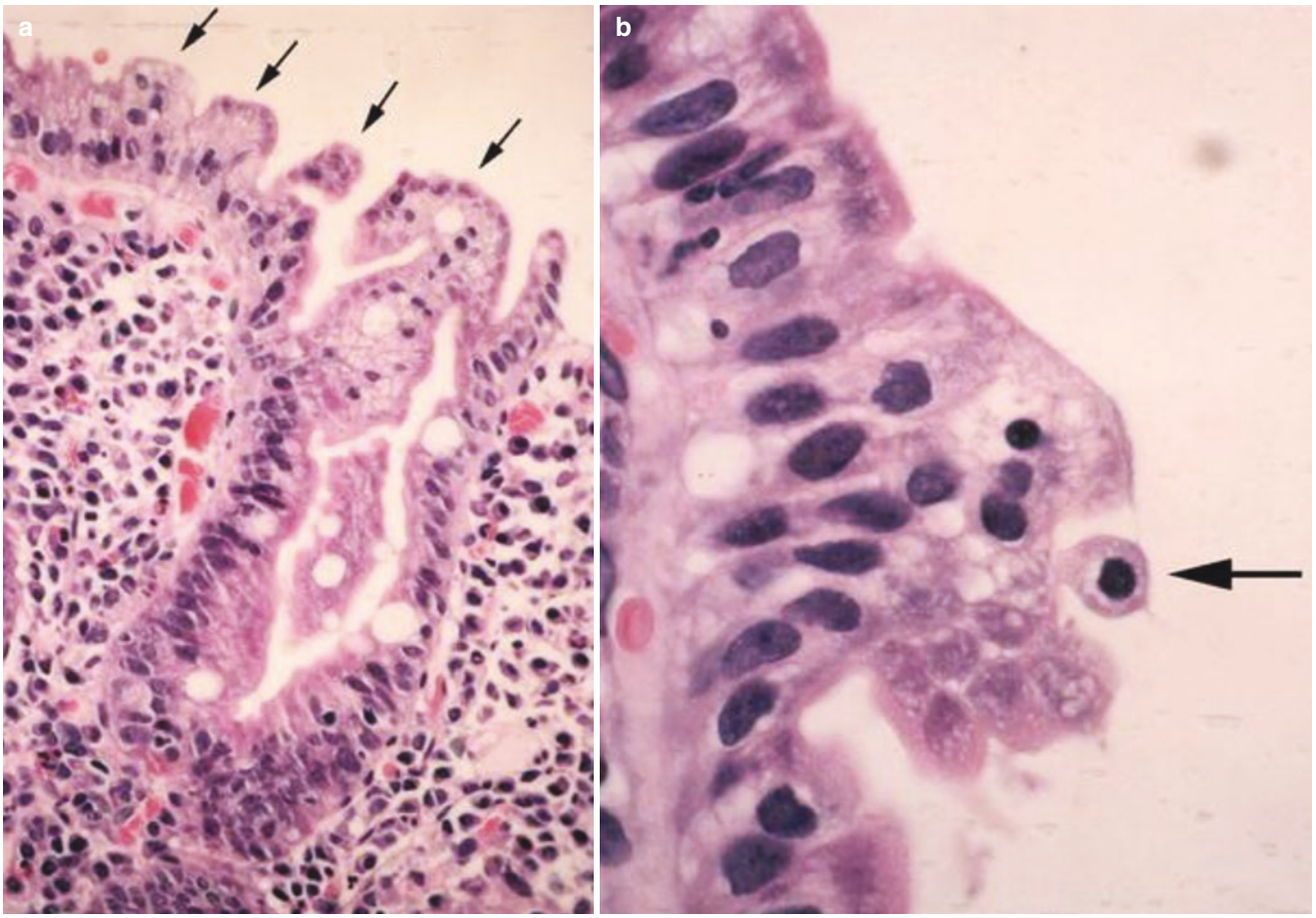

Fig. 1.6 (**a**) Numerous tufts of enterocytes (*) on the mucosal surface of the duodenum. (**b**) A characteristic tear-drop-shaped structure (*arrow*) in an epithelial tuft (H&E stain; original magnification: a – ×80; b – ×400) [58] (Reprinted from Springer and European Journal of Pediatrics, El-Matary et al. [91], Fig. 1, with kind permission from Springer Science and Business Media)

Cases totally dependent on total parenteral nutrition are candidates for intestinal transplantation.

Tufting enteropathy is often associated with other pathologies related to epithelial cells or malformations. More than 60% of patients have punctate keratitis [97] or other eye diseases [98], associated with other changes, such as nostril atresia, esophageal atresia, or absence of the anus [79].

In some cases where there was a risk of liver failure as a result of TPN, an intestinal or combined liver / intestinal transplant was performed, with satisfactory results in a case where the transplant was performed before aggravation. Repeated biopsies are sometimes required for diagnosis.

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The Spectrum of Autoimmune Enteropathy

2

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Introduction

Chronic, unexplained diarrhea in children younger than 3 months old was first characterized as "intractable diarrhea" [1]. The term "protracted diarrhea" was later used to describe infants with frequent and loose stools severe enough to often require parenteral alimentation as nutritional support [2]. The differential diagnosis of enteropathies in infancy and childhood includes inherited epithelial and congenital transport defects, enzymatic deficiencies, and allergic enteropathy (Table 2.1).

The most frequent diagnosis in children with protracted diarrhea is autoimmune enteropathy [3, 4]. It is a rare, immune-mediated disorder starting usually within the first months of life. The age of onset is between 1 month and 5 years (median age 17 months) [5], but late-onset adult forms have been also reported [6–9]. The disease was first described by Walker-Smith et al. in 1982 in a male child with clinical features of celiac disease and villus blunting unresponsive to gluten-free diet [10] and represents a heterogeneous group of disorders rather than a discrete entity. The incidence is estimated at less than 1 in 100000 infants. The diagnostic criteria are debatable, but the presence of circulating anti-enterocyte antibodies and the lack of immunodeficiency have been proposed as the hallmark features of autoimmune enteropathy [5, 11]. The latter criterion has been challenged by clinical experience and better understanding of the immunology of autoimmunity and self-tolerance [12].

Table 2.1 Differential diagnosis of diarrhea in infancy and childhood

Transport defects and enzymatic deficiencies
Disaccharidase deficiency
Sodium–hydrogen exchanger (congenital sodium diarrhea)
Chloride–bicarbonate exchanger (chloride-losing diarrhea)
Sodium-glucose cotransporter (glucose–galactose malabsorption)
Lysinuric protein intolerance
Chylomicron retention disease
Abetalipoproteinemia
Ileal bile acid receptor defect
Enterokinase deficiency
Inherited epithelial defects and villous atrophy
Enterocrine cell dysgenesis
Microvillus inclusion disease
Autoimmune enteropahty
IPEX syndrome
Tufting enteropathy
Lymphangectasia
Celiac disease
Allergic enteropathy/eosinophilic enteritis
Infectious/post-infectious enteropathy
Other
Idiopathic
Acrodermatitis enteropathica
Metabolic diseases
Tumors

Autoimmune enteropathy is characterized by variable clinical expression, ranging from isolated gastrointestinal involvement to severe systemic disease [13, 14]. Patients diagnosed with the disease often exhibit extra-intestinal manifestations of autoimmunity, in contrast to those with tufting enteropathy and microvillus inclusion disease [15]. Based on a genetic approach combined with immunological evaluation three different forms of autoimmune enteropathy have been proposed:

1. A predominately or isolated gastrointestinal form of autoimmune enteropathy with typical anti-enterocyte antibodies in both sexes
2. A systemic X-linked form of autoimmune enteropathy associated with different endocrinopathies, hematologi-

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cal symptoms, and severe eczematic skin disease, known as immune dysregulation, polyendocrinopathy, and autoimmune enteropathy X-linked syndrome (IPEX) occurring only in males
3. An IPEX-like form, a priori FOXP3-independent occurring in both sexes

IPEX and APECED syndromes (APR-1/autoimmune phenomena, polyendocrinopathy, candidiasis, and ectodermal dystrophy) are systemic forms of autoimmune enteropathy [16].

Diagnosis

The diagnostic criteria for autoimmune enteropathy were originally proposed by Unsworth and Walker-Smith et al. and included (a) protracted diarrhea and severe enteropathy with small-intestinal villous atrophy, (b) no response to exclusion diets, (c) evidence of predisposition to autoimmune disease (presence of circulating enterocyte antibodies or associated autoimmune disease), and (d) no severe immunodeficiency [17]. A more recent adult study proposed the updated criteria and now the diagnosis is established when all of the criteria are present (Table 2.2). The disease should be considered in the differential diagnosis in all patients presenting with severe, unexplained diarrhea requiring parenteral nutritional support particularly in infants, since autoimmune enteropathy is the most common cause of protracted diarrhea in infancy [3]. The endoscopic examination with small bowel biopsy is the cornerstone of investigation.

The diagnostic work-up should also include information regarding the birth and family history and the time of onset of diarrhea. The disease is characterized by secretory diarrhea, nonresponsive to bowel rest. Most of the affected infants have no history of gluten ingestion at the time of presentation. Furthermore, the lack of response to a gluten-free diet points toward autoimmune enteropathy [5, 11, 18].

Serum immunoglobulin assays show normal IgM and decreased IgG attributed to protein-losing enteropathy. IgA is often within normal range, but IgA deficiency associated with villous atrophy has been also reported in autoimmune enteropathy. T- and B-cell function tests, the lymphocytic subsets, and polymorphonuclear cell counts are generally normal. Anti-smooth muscle, anti-nuclear, and anti-thyroid microsomal autoantibodies have been identified during the disease [5, 8, 19, 20].

Determination of fecal inflammatory markers, like fecal calprotectin, is a simple method that is helpful in distinguishing constitutive intestinal epithelial disorders, such as microvillus atrophy and epithelial dysplasia from immune-inflammatory etiologies such as autoimmune enteropathy and inflammatory colitis. It has been proposed that the dramatically increased levels of fecal calprotectin in neonates and infants with immune-inflammatory disorder can distinguish these disorders from constitutive epithelial disorders with 100% specificity [21].

Table 2.2 Diagnostic criteria for AIE

Diagnostic criteria for AIE by Unsworth and Walker-Smith [17]	Updated diagnostic criteria for AIE by Akram et al. [14]
1. Protracted diarrhea and severe enteropathy with small-intestinal villous atrophy 2. No response to exclusion diets 3. Evidence of predisposition to autoimmune disease (presence of circulating enterocyte antibodies or associated autoimmune disease) 4. No severe immunodeficiency	1. Chronic diarrhea (>6 weeks) 2. Malabsorption 3. Small bowel histology showing partial or complete villous blunting, deep crypt lymphocytosis, increased apoptotic bodies, minimal intraepithelial lymphocytosis 4. Exclusion of other causes of villous atrophy, including celiac disease, refractory sprue, and intestinal lymphoma 5. Presence of anti-enterocyte and/or anti-goblet cell antibody supports the diagnosis and sometimes correlates with disease improvement, but is not required to make the diagnosis

Clinical Presentation

Chronic, secretory diarrhea refractory to bowel rest that leads to dehydration, electrolyte abnormalities, malabsorption, and severe weight loss is the typical clinical presentation of autoimmune enteropathy. Diarrhea usually begins between 2 and 4 weeks of age, and the secretory component can be delayed for a few months [3, 11, 22]. The symptoms can be debilitating, unresponsive to restriction of diet, and the disease is potentially life-threatening. The establishment of the diagnosis is crucial in order to ensure optimal treatment. Patients typically require immunosuppressive therapies and total parenteral nutrition for electrolyte balance and nutritional support [17, 23].

Even though the mucosal abnormality is primarily confined to the small intestine, the term "generalized autoimmune gut disorder" has been used to describe the association between autoimmune enteropathy and autoimmune colitis [8]. Emerging evidence suggests that autoimmune enteropathy can be a manifestation of a more diffuse autoimmune disorder of the gastrointestinal system, comprising gastritis, colitis, hepatitis, and pancreatitis with positivity of a variety of autoantibodies, including anti-parietal, anti-goblet cell, and anti-smooth muscle antibodies [6, 19, 24, 25].

Furthermore, the involvement of extraintestinal organs can be present during the course of the disease. Multisystem extra-intestinal manifestations include endocrine, renal,

pulmonary, hematologic, and musculoskeletal. Hypothyroidism with interstitial fibrosis and lymphocytic infiltration of the thyroid gland, membranous glomerulonephritis and nephrotic syndrome, interstitial pneumopathy, periportal fibrosis and bronchitis, hemolytic anemia, rheumatoid arthritis, thymoma, and dermatitis/atopic eczema have all been reported [5, 11, 19, 26, 27, 28]. Severe forms of AIE can be associated with identified syndromes, namely IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) and APECED (autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy) syndrome [11].

Thymus plays a key role in the deletion of potentially self-reactive clones of T cells. The association between autoimmune enteropathy and thymoma has been described in both pediatric and adult patients and provides further evidence about the role of thymoma and the development of autoimmunity [27, 28].

Pathogenesis

The underlying immunologic and molecular mechanisms in autoimmune enteropathy have not yet been fully elucidated and are widely debatable. However, it has been established that an autoimmune response is involved in the pathogenesis of the disease. Thymus plays a key role and orchestrates a healthy immune system. The intrathymic maturation of T lymphocytes is crucial for the deletion of potentially self-reactive clones of T cells. The dysfunction of the thymus results in the nondeletion and presence of self-reactive T cells that can induce the expansion of anti-self B cells [11, 29, 30].

In autoimmune enteropathy, the gut is the site where the autoimmune reaction takes place and is mediated by the activation of self-reactive T cells locally, resulting in the typical histological lesions. In normal states, the expression of human leukocyte antigen (HLA) class II molecules on the enterocyte surface is crucial in establishing and maintaining the oral tolerance as the epithelial cells present exogenous peptides to the clonotypic T-cell receptors. The overexpression of HLA-DR antigens in enterocytes and the inappropriate expression of HLA class II molecules in the crypt epithelium of the proximal small intestine in children with autoimmune enteropathy have been reported [15, 31]. The overexpression of HLA class II molecules results in the proliferation of CD4+ and CD8+ T lymphocytes [8, 15, 32].

An increase in the levels of CD4+ and CD8+ T lymphocytes in the lamina propria in subjects affected by autoimmune enteropathy provides further evidence that the T cells are involved in the pathogenesis of the disease [33, 34]. The intestinal T lymphocytes cause damage to the enterocytes by exerting direct cytotoxicity, via the production of lymphokines or through an antibody-dependent cytotoxicity resulting in cellular apoptosis [32, 35, 36].

A variety of circulating auto-antibodies such as antibodies against gastric parietal cells, pancreatic islets, glutamic acid decarboxylase, insulin, smooth-muscle, endoplasmic reticulum, reticulin, gliadin, adrenal cells, nuclear antigens, DNA, thyroglobulin, and thyroid microsomes has been detected in patients with autoimmune enteropathy [7, 17]. The presence of antibodies against goblet cells, enterocytes, and colonocytes is supportive of the diagnosis. These antibodies are directed against components of the intestinal brush border membrane, with an increasing intensity from the crypts toward villus tip [5, 13]. However, they are neither diagnostic nor specific for the disease and have been also identified in other disorders such as the cow's milk allergy, inflammatory bowel disease and in adults with HIV infection. Moreover, the appearance of the autoantibodies after the onset of the mucosal damage, the lack of correlation between the titer and the histological severity, and their disappearance after treatment, but before the complete mucosal restoration support the hypothesis that these antibodies are most likely a secondary event in the pathogenesis of the disease in response to bowel injury [10, 37–39].

The nature of the gut antigen that elicits the immune response and results in the alteration of the intestinal permeability has been extensively investigated. A 55kD protein located in both the gut and renal epithelial cells that reacted with serum autoantibodies was first identified by Colletti et al. in 1991 in a patient with complicated presentation of autoimmune enteropathy with small bowel and glomerular involvement [40]. A few years later, a 75kD autoantigen that is distributed through the whole intestine and the kidney was recognized in patients with X-linked autoimmune enteropathy associated with nephropathy, as reported by Kobayashi et al. [41]. Autoantibodies against this 75kD autoantigen, known as harmonin, are specific to IPEX patients [42, 43, 44]. Kobayashi et al. also identified in a proportion of patients with IPEX the autoantibodies against villin; the actin-binding 95 kDa protein involved in the organization of actin cytoskeleton in the brush border of epithelial cells was described as an additional target of autoantibodies in a proportion of patients with IPEX [43, 45]. The intestinal auto-antigen in autoimmune polyendocrine syndrome type 1 (APECED) is tryptophan hydroxylase, which is mainly present in the enterochromaffin cells of the mucosa [46].

Emerging evidence has pointed toward an uncontrolled inflammatory reaction caused by the disturbance of the effector–regulatory T-cell interaction and leading to the production of autoantibodies, such as anti-enterocyte antibodies [47]. The understanding of the underlying molecular mechanism and the identification of the genetic defect in IPEX was achieved due to the clinical similarities between

scurfy mice and boys with the disease. Scurfy mice are naturally occurring X-linked mutants that present with massive lymphoproliferation, diarrhea, intestinal bleeding, scaly skin, anemia, thrombocytopenia, and hypogonadism [48]. Based on the observation that the disease-causing mutation in scurfy mice was on the X chromosome, the human IPEX locus was identified on chromosome Xp11.23-q13.3 and the gene was named FOXP3. It comprises of 11 exons which encode the FOXP3 protein or scurfin, a 48 kDa protein of the forkhead (FKH)/winged helix transcription factor family that is predominantly expressed in CD4+CD25+ T cell with regulatory function, at significantly lower levels in CD4+CD25– T cells and not at all in CD8+ or B220+ cells [49–52].

Increasing experimental evidence has shown that scurfin is implicated in the thymic maturation of T cells that are designated to acquire regulatory function. CD4+CD25+ Tregs represent a small subset (5–10%) of CD4+ T helper cells in humans and mice. Studies on CD4+CD25+ T cells from IPEX patients with the use of anti-CD127 have shown that FOXP3 plays a crucial role in the generation of functional T regulatory cells (Tregs) and intact FOXP3 is indispensable for the development of fully functional Tregs, whereas FOXP3 with amino acid substitutions in the FKH domain is sufficient for the generation of functionally immature Tregs [53]. Tregs are potent immunosuppressive cells of the adaptive immune system. The suppressive mechanisms for Treg cells include CTLA4 engagement of B7 molecules on target cells; expression of immunosuppressive cytokines such as IL-10, TGF-β, and IL-35; cytotoxicity of target cells through the perforin/granzyme pathway; induction of indoleamine 2,3-dioxygenase (IDO); and the catabolism of tryptophan in target cells, as well as consumption of adenosine by expression of CD73 and competition with effector T cells for IL-2 since Treg cells constitutively express the high affinity IL-2 receptor CD25 [54]. The loss of the regulatory function of T lymphocytes with subsequent uncontrolled inflammatory reaction is also implicated in the pathogenesis of IPEX syndrome.

FOXP3 has deoxyribonucleic acid (DNA) binding activity, and due to its structure may serve as nuclear transcription factor and act as a repressor of transcription and regulator of T-cell activation [55, 56]. The transcription of a reporter containing a multimeric FKH binding site is repressed by intact FOXP3. Such FKH binding sites are located adjacent to nuclear factor of activated T cells (NFAT), regulatory sites in various cytokine promoters such as IL-2, or granulocyte-macrophage colony-stimulating factor enhancer. Therefore, intact scurfin protein appears to be capable to directly repress NFAT-mediated transcription of the IL-2 gene in CD4+ cells upon activation [57].

Data from animal models with transgenic induction of FOXP3 have shown that the overexpression of scurfin in normal mice leads to a tremendous suppression of immune functions, whereas the depletion of Tregs in healthy mice results rapidly in the development of different T-cell-mediated autoimmune disorders, similar to scurfy in mice or IPEX in humans that go in complete remission upon reconstitution with Treg cells [58, 59].

The three domains that are crucial for the function of FOXP3 are the C-terminal region, which contains the forkhead domain that directly binds DNA regions, the central domain with a zinc finger and leukine zipper that promotes the oligomerization of the FOXP3 molecule, and the repressor domain located in the N-terminal region that binds the NAFT [60, 61]. Genetic screening on X chromosome in patients with IPEX revealed that the majority of mutations cluster primarily within the FKH domain and the leukine zipper within the coding region of the FOXP3 gene causing potentially absent FOXP3 protein expression or a protein product with loss of function [13, 60].

Histopathology

Histologic evaluation of the small bowel in typical autoimmune enteropathy reveals partial or total villous blunting/atrophy and crypt hyperplasia. In addition, there is a marked infiltration of the lamina propria by mixed inflammatory cells with a prominence of mononuclear cells, including T lymphocytes [15]. Apoptotic bodies and intraepithelial lymphocytes are present in the crypt epithelia. Most cases show a relative paucity of surface lymphocytosis in contrast to celiac disease. The lymphocytic infiltration of the intestinal mucosa is constituted by CD4-CD8 T lymphocytes and macrophages. Goblet, Paneth, and/or enterochromaffin cells may be reduced in number or absent. Cryptitis and crypt abscesses have been reported in severe autoimmune enteropathy. Crypt enterocytes commonly show an increased expression of HLA-A, -B, -C molecules [8, 62, 63] (Table 2.3) (Fig. 2.1).

Autoimmune enteropathy primarily involves the small bowel with the histologic lesions being most prominent in the proximal small intestine. However, changes have been

Table 2.3 Histological findings in autoimmune enteropathy

1. Partial or total villous blunting/atrophy and crypt hyperplasia.
2. Marked infiltration of mononuclear cells, including activated T lymphocytes in the lamina propria.
3. Apoptotic bodies and intraepithelial lymphocytes present in the crypt/gland epithelia, but relatively paucity of surface lymphocytosis.
4. Crypt abscesses in severe autoimmune enteropathy.
5. Increased expression of HLA-A, -B, -C molecules in crypt enterocytes.

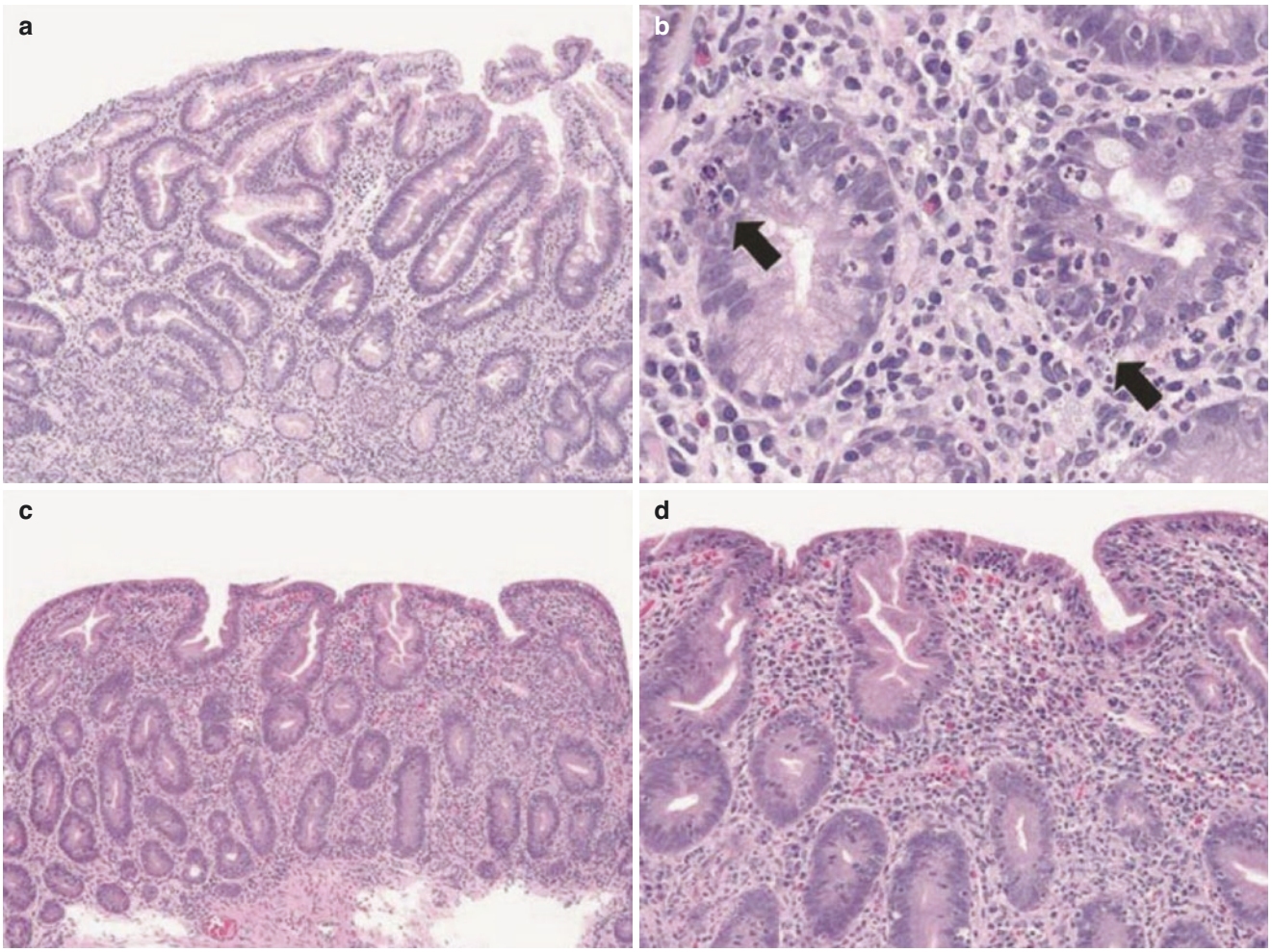

Fig. 2.1 (**a**, **b**, low and high magnifications, respectively) In some cases of pediatric autoimmune enteropathy, the small intestinal biopsies show cryptitis and crypt abscesses that may obscure the salient finding of autoimmune enteropathy, crypt apoptosis (arrows). There is also an absence of Paneth cells. (**c**, **d**) As described in adult patients, small intestinal biopsies can demonstrate a combination of both autoimmune enteropathy and sprue-like histologic findings, characterized by severe villous blunting, marked intraepithelial lymphocytosis, diffuse mononuclear inflammatory infiltrate, and prominent crypt apoptosis. Of note, goblet cells are lacking within this specimen

also described in the esophagus, stomach, and colon in both pediatric and adult patients supporting the hypothesis for a diffuse disease process involving the entire gastrointestinal tract.

Recent reports describe the infiltration of the squamous epithelium by lymphocytes or eosinophils in the esophagus. Gastric biopsies can show features of chronic nonspecific gastritis with or without reactivity. Atrophic gastritis, intestinal metaplasia, and glandular destruction have been also described. There may be increased apoptosis of glandular epithelium [6, 64]. The colonic morphological lesions vary from mild active colitis with inflammatory cell infiltration to severe, diffuse, chronic colitis with goblet cell depletion, Paneth cell metaplasia, distortion of crypt architecture, and crypt abscess formation. An increase in intraepithelial lymphocytes has been also described [25, 64].

Treatment

Early recognition and accurate diagnosis of autoimmune enteropathy is mandatory to ensure the optimal treatment. The disease is characterized by life-threatening diarrhea often nonresponsive to bowel rest. Total parenteral nutrition (TPN) represents an important step in the management of autoimmune enteropathy for nutritional support, adequate rehydration, and optimal growth [11, 14, 65]. However, the pediatric patients are not always TPN-dependent during the course of the disease [66]. When the gastrointestinal involvement is less severe, elemental or low carbohydrate containing formula is recommended to promote enteral delivery of nutrients and calories. The potential tolerance to enteral feeds and the concomitant inflammatory changes affecting the colon make small bowel transplantation not an ideal treatment option for autoimmune enteropathy [17, 67].

Long-term immunosuppression is the mainstay of treatment for the disease. Standard immunosuppressive therapies include corticosteroids, cyclosporine, azathioprine, and 6-mercaptopurine. Steroids in the form of prednisolone or budesonide are often needed to induce remission. However, the disease can be refractory to steroids or diarrhea recurs when they are tapered [68].

Since the early 1990s pediatric patients with autoimmune enteropathy have been successfully treated with oral cyclosporine A. Studies have shown that a relatively low drug level (50 ng/mL) led to improvement in growth, intestinal carbohydrate absorption, and small bowel histology. However, a number of patients do not respond to the medication and a possible reason is the inefficient absorption of the oral compound due to the underlying chronic enteropathy [69, 70].

Tacrolimus has been used as a therapeutic treatment option with beneficial effects in a variety of autoimmune diseases, including autoimmune hepatitis, primary sclerosing cholangitis, and steroid-refractory nephrotic syndrome. Its mechanism of action is similar to cyclosporine. Both drugs block the gene activation for cytokine production by inhibiting the antigenic response of helper T lymphocytes, suppressing interleukin and interferon-γ [22]. Bousvaros et al. first used tacrolimus as an alternative therapy for autoimmune enteropathy and concluded that it can be efficacious if other immunosuppressive regimens fail. Clinical improvement occurred between 1 and 4 months once therapeutic levels were achieved, and serum drug levels were obtained between 5 and 15 ng/mL. Tacrolimus' absorption is less dependent on mucosal integrity compared to cyclosporine; however, mucosal healing improves its absorption, and the dosage should be adjusted to achieve the desired blood levels of the drug. The need for long-term or lifelong treatment with tacrolimus in autoimmune enteropathy necessitates baseline and frequent monitoring in order to prevent potential complications including nephrotoxicity, neurotoxicity, increased predisposition to infections, and lymphoproliferative disease [71].

The combination of tacrolimus and infliximab has been proven successful in controlling the inflammation in severe autoimmune enteropathy in both pediatric and adult patients. The synergistic effect of these agents is based on the different aspects of the immune system on which they act. Emerging evidence support that infliximab itself is a highly effective tool for achieving clinical remission and restoring small bowel villous architecture in autoimmune enteropathy. The drug has been introduced because of its TNF-α antagonistic effect since high levels of this cytokine are being produced by intestinal intraepithelial T lymphocytes of patients with autoimmune enteropathy. The response to infliximab is usually rapid and the quality of life of the patients improves dramatically. However, it has been recognized that aggressive immunosuppressive treatment carries a potential risk of life-threatening hypersensitivity reactions and malignancies that should be also considered [17, 72, 73].

Additional immunosuppressive therapies have been used in autoimmune enteropathy. Cyclophosphamide is an alkylating agent related to nitrogen mustard and is a potent immunosuppressive agent used in bone marrow transplantation (BMT) conditioning regimens [74]. Low dose oral cyclophosphamide led to resolution of the intestinal symptoms in a teenage boy with total villous atrophy, selective immunoglobulin A deficiency, and anti-epithelial cell antibodies [75]. However, the use of cyclophosphamide in doses up to 3 mg/kg/d has not always been successful in the management of the disease [40, 76]. Remission of symptoms and improvement of intestinal histopathology was reported in an infant with severe autoimmune enteropathy with a single course of high-dose intravenous cyclophosphamide, approximately 20 times greater than those previously used [77].

Myco-phenolate mofetil (MMF) has been proposed as an alternative therapeutic option after the successful induction of remission and the improvement of the intestinal absorption and linear growth in an infant with autoimmune enteropathy and concomitant factor V Leiden defect [78].

Rapamycin is a macrolide compound and has immunosuppressive and antiproliferative properties due to its ability to inhibit mTOR (mammalian target of rapamycin) [79]. Rapamycin was found to be beneficial and appeared to be superior to calcineurin inhibitors when used as an immunosuppressive therapy in IPEX patients [80].

The successful use of adalimumab, an *antitumor necrosis factor*-alpha monoclonal antibody administered subcutaneously, has been reported in adult cases of autoimmune enteropathy [81, 82]. Other biologics, including vedolizumab (an anti-α4β7 integrin) and abatacept, have also been used in adult cases of autoimmune enteropathy [83, 84, 85].

IPEX Syndrome

IPEX is a rare disease and represents a systemic form of autoimmune enteropathy characterized by immune dysfunction, polyendocrinopathy, enteropathy, and X-linked inheritance. The prevalence of the syndrome remains unknown.

IPEX is inherited in males via an x-linked recessive manner and results from mutations in the *FOXP3* gene located at the Xp11.23-q13.3 locus [51]. At least 70 distinct *FOXP3* mutations have been reported, although identical *FOXP3* mutations can cause different phenotypes in patients [42].

Even though the severity of symptoms is variable, the most common feature of IPEX is the involvement of pancreas

and thyroid with an early onset. Glucose intolerance can be present at birth and insulin-dependent diabetes mellitus begins often during the first year of life as a result of the complete inflammatory destruction of the pancreatic islet cells prior to the intestinal symptoms [86]. Thyroiditis presents most commonly as hypothyroidism requiring substitutive therapy but may also present in the form as hyperthyroidism [13, 65].

The gastrointestinal involvement in children with IPEX syndrome includes severe, secretory diarrhea that can be bloody or mucousy and generally worsens after the breastfeeding is changed to formula [87, 88]. Protein-losing enteropathy with hypoalbuminemia and a markedly increased clearance of a-1 antitrypsin are indicators of a poor prognosis [47]. Diarrhea often persists despite bowel rest, and total parenteral nutrition is required for nutritional support.

In addition to the endocrinopathies and the gastrointestinal involvement, the basic triad of IPEX syndrome includes chronic dermatitis, most commonly in the form of eczema. In addition, other immune-mediated dermatological disorders like alopecia, pemphigoid nodularis, psoriasiform dermatitis, or onychomycosis-like lesions have been described [87, 89, 90]. The majority of boys with classical IPEX syndrome develop autoimmune hematologic disorders, such as Coombs-positive hemolytic anemia, neutropenia, or thrombocytopenia with anti-platelet antibodies. Renal involvement affects approximately 30% of the cases and presents as tubulopathy, nephrotic syndrome, or glomerulonephritis. Autoimmune hepatitis is also common. The clinical spectrum of the syndrome includes neurological manifestations, such as seizures and developmental delay. The majority of patients do not experience recurrent infections, as the disease itself does not impair the body's response to pathogens. However, IPEX syndrome carries a higher risk of infections due to the disruption of skin or gut barrier, as well as the need for immunosuppressive regimen [42] (Table 2.4).

The small bowel histopathology of pediatric subjects with IPEX syndrome can range from a graft-versus-host disease-like pattern with complete villous atrophy, mild inflammation of the lamina propria, apoptotic bodies, crypt abscesses, and loss of goblet cells (as described above) to a more celiac-disease-like pattern with partial villous atrophy, moderate inflammation of the lamina propria, an increase in intraepithelial lymphocytes, and crypt hyperplasia. Moreover, a patient with anti-goblet cell antibodies demonstrated partial villous atrophy, moderate inflammation of the lamina propria, an increase in intraepithelial lymphocytosis, and complete lack of goblet cells [91]. Depending on the organs involved in IPEX syndrome, the typical lymphocytic infiltration can also be present in thyroid, liver, skin, brain, or pancreas with complete destruction of islet cells [65, 92].

Table 2.4 Multisystemic disorders in IPEX syndrome

IPEX syndrome
Endocrine: glucose intolerance, insulin-dependent diabetes mellitus, hypo/hyperthyroidism
Gastrointestinal: secretory diarrhea, protein-losing enteropathy, autoimmune hepatitis
Skin: eczema, alopecia, pemphigoid nodularis, psoriasiform dermatitis
Hematologic disorders: autoimmune hemolytic anemia, neutropenia, thrombocytopenia
Renal: glomerulonephritis, tubulopathy, nephrotic syndrome
Neurological manifestations: seizures, developmental delay
Other: susceptibility to infections, reactions to vaccines

The syndrome should be considered in young males presenting with protracted diarrhea with villous atrophy and failure to thrive combined with diabetes mellitus and/or hypothyroidism and skin manifestations. Anti-harmonin autoantibodies (HAA) and anti-villin autoantibodies (VAA) are useful for the preliminary screening of patients for IPEX syndrome. HAA has a high specificity for IPEX syndrome. As villin is highly immunogenic, VAA can be negative in IPEX patients with enteropathy and positive in other autoimmune diseases [42, 43]. The definite diagnosis of IPEX is based on genetic studies and mutation analysis of the FOXP3 gene [93]. Immunocytochemical staining of FOXP3 molecule in bowel biopsies has been proposed as a potential screening test [94].

Given the potential serious side effects and the limited efficacy for long-term remission of immunosuppressive medication, there is a need for new therapeutic approaches for IPEX and autoimmune enteropathy. The most promising is in the form of hematopoietic stem cell transplantation (HSCT). A recent literature review of case reports and case series for HSCT in IPEX patients found 28 cases of pediatric patients who had HSCT between 4 months and 16 years of age. The source of hematopoietic cells was bone marrow for 20 patients and peripheral blood for 8 patients, with successful results in 23 of the 28 patients. Of the five unsuccessful cases, one required re-transplantation. Death was reported in 5 out of 28 patients (21%). Complications included infections, autoimmune cytopenia, pancreatitis, and GVHD [95].

A recent retrospective multicenter study by the group Barzaghi et al. evaluated the disease onset, progression, and long-term outcome of IPEX patients receiving HSCT or prolonged immune suppression. Out of 96 patients in the study, 34 received immunosuppression, 58 underwent HSCT, and 4 did not require immunosuppression or HSCT. HSC sources included bone marrow (35/58), peripheral blood stem cells (12/58), and cord blood (13/58). Survival rates at 15 years among patients undergoing HSCT were lower, but not significantly, compared to patients who only received long-term immunosuppression (73.2% vs 86.8%; $p = 0.055$). This was due to the high mortality rate within 2.5 years

following transplantation, with no additional mortality rate for patients who survive over 2.5 years after transplant up to 15 years later. IPEX patients with higher organ involvement score (severe organ impairment) at HSCT had the lowest chance of survival. Non-transplanted patients had persistent disease progression that contributed to increased mortality over time [80].

Interestingly, the conditioning regimen was itself effective in controlling symptoms in some patients [55, 97] and a reduced-intensity conditioning regimen prior to engraftment resulted in more FOXP3 T regulatory cells than more aggressive conditioning [96, 98]. Beneficial effect on the pancreatic function and recovery of diabetes mellitus was achieved in some cases; however, the islet damage is often permanent at the time of bone marrow transplantation [86]. Insulin for diabetes and hematic transfusions are often needed for the symptomatic management of IPEX syndrome.

IPEX-Like Syndromes

A number of other gene defects that give rise to an IPEX-like phenotype have been discovered, including loss of function mutations in CD25 (IL2RA), STAT5b, ITCH, gain of function mutations in STAT1, gain of function mutations in STAT 3, CTLA4, and LRBA (54, 99). *CD25* is the α-chain of the interleukin 2 (IL-2) receptor. Deficiency of CD25 leads to defects in responsiveness to IL-2 which results in widespread immune dysregulation. Patients with CD25 deficiency have clinical similarities to IPEX patients, with symptoms including severe diarrhea, insulin-dependent diabetes mellitus, autoimmune thyroiditis, early onset eczema, alopecia universalis, and autoimmune enteropathy. However, CD25 deficiency is distinguished by a profound susceptibility to viral, fungal, and bacterial infections. CMV pneumonitis, enteritis, and EBV lymphoproliferative disease, oro-esophageal candidiasis, and adenoviral gastroenteritis have been reported in patients with CD25 deficiency [54].

Signal transducer and activator of transcription 5 (STAT5) consists of two highly related proteins, STAT5A and STAT5B, which are encoded by two separate genes but are 90% identical at the amino acid level. STAT5 proteins are involved in cytosolic signaling and in mediating the expression of specific genes. Defects in STAT5b are inherited in an autosomal recessive manner. Many of the features of STAT5b deficiency, including eczema, chronic diarrhea, thyroiditis, and increased susceptibility to infections, are similar to findings in CD25 deficiency. This is likely due to the fact that IL-2 signals are transmitted via STAT5 [54, 99]. The severity of disease in STAT5b deficiency is less severe compared to CD25 deficiency, probably because STAT5a may substitute some of the functions of STAT5b deficiency [54].

Autosomal dominant heterozygous gain-of-function (GOF) mutations in STAT1, which is associated with chronic mucocutaneous candidiasis, have been found by Uzel et al. in five patients with IPEX-like symptoms. All five patients presented with eczema and enteropathy. Endocrinological manifestations in these patients include type 1 diabetes, hypothyroidism, and growth hormone insufficiency. Four patients had mucocutaneous candidiasis. All five patients suffered from recurrent infections including respiratory infections, herpes virus infections, and blood-borne infections. The patients also suffered from cardiovascular problems, including vascular aneurysms, calcification, and hypertension [100].

Children within an extended Old Order Amish kindred presented with developmental delay, dysmorphic features, failure to thrive organomegaly, and multisystem autoimmune diseases, including autoimmune enteropathy, with autosomal recessive inheritance being apparent in this extended family in which prominent consanguinity was noted. Human ITCH E3 ubiquitin ligase deficiency was recognized as the underlying cause of this syndromic multisystem autoimmune disease. ITCH deficiency results in abnormal T helper cell differentiation and failure of T cell anergy induction. Genetic mapping in patients revealed a truncating mutation in the ITCH gene [101, 102].

Heterozygous germline GOF mutations in STAT3 have been associated to multiorgan autoimmune manifestations (i.e., type 1 diabetes, enteropathy, interstitial lung disease, cytopenia, and hypothyroidism), lymphoproliferation, short stature, and recurrent infections [103].

CTLA4 or CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), also known as CD152, is expressed by activated T cells and transmits an inhibitory signal to T cells. CD28, on the other hand, is expressed on T cells that provide co-stimulatory signals required for T-cell activation and survival. Both molecules compete for ligands CD80 and CD86 on antigen-presenting cells. CTLA-4 binds CD80 and CD86 with greater affinity and avidity than CD28 thus enabling it to outcompete CD28 for ligand binding [104]. Lipopolysaccharide-responsive beige-like anchor protein (LRBA) is a cytosolic protein which blocks the transport of CTLA-4 (that are constitutively internalized from the surface of Treg cells) to lysosomes for removal, thereby maintaining the amount of CTLA-4 trafficked back to the cell surface [105]. This is important as CTLA-4 plays a role in the inhibition of self-reacting T cells after antigen recognition. Patients with CTLA4 mutations suffer from recurrent respiratory tract infections, hypogammaglobulinemia, autoimmune cytopenia, autoimmune enteropathy, and granulomatous infiltrative lung disease [104]. LRBA deficiency presents as a syndrome of autoimmunity, lymphoproliferation, and humoral immune deficiency, with clinical manifestations including immune dysregulation, organomegaly, recurrent infections, hypo-

gammaglobulinemia, idiopathic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia (AIHA), autoimmune enteropathy, granulomatous-lymphocytic interstitial lung disease, and type I diabetes [50].

APECED Syndrome

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), also known as autoimmune polyendocrine syndrome type 1 (APS-1), is a rare, autosomal recessive disease. APECED is caused by biallelic mutations in the autoimmune regulatory or AIRE gene in chromosome 21q22.3 [106, 107]. AIRE is expressed in medullary thymic epithelial cells and promotes expression of tissue specific antigens to be displayed to developing T cells. With this unique antigen presentation to developing T cells, autoreactive T cells with affinity for self-proteins either die by apoptosis or become forkhead box P3 (FoxP3) expressing T regulatory cells (Tregs). When AIRE is nonfunctional or absent, autoreactive T cells escape to the general circulation and peripheral lymphoid organs where they can cause autoimmune reactions and the disease APS-1 [108, 109]. The understanding of the underlying pathophysiology of this monogenic disease has provided important information on the pathogenesis of organ-specific autoimmunity and T cell selection.

APECED has been reported at a relatively higher prevalence in genetically isolated populations like Finns, Sardinians, and Iranian Jews [110–113]. The classical triad of APECED consists of Addison's disease, hypoparathyroidism, and chronic mucocutaneous candidiasis, and two of them are required for the diagnosis. However, several other endocrine and non-endocrine manifestations accompany the pathognomonic triad.

The onset of APECED is usually in childhood with chronic mucocutaneous candidiasis occurring as early as infancy the first symptoms occurring on average at the age of five [114]. The wide spectrum of the clinical manifestations is attributed to a variable pattern of destructive autoimmune reaction mediated by specific antibodies that can attack any tissue or organ [115, 116]. Tryptophan hydroxylase (TPH) is an enzyme involved in the synthesis of neurotransmitters in the nervous system and in the gastrointestinal endocrine cells. The presence of autoantibodies against this enzyme is associated with autoimmune enteropathy in APECED [117–120]. The gastrointestinal involvement also includes diarrhea, constipation, autoimmune hepatitis, chronic atrophic gastritis, or autoimmune gastritis with pernicious anemia [121]. Exocrine pancreatic failure results in malabsorption and steatorrhea in a small subgroup of patients [114].

Patients affected by at least two of the triad of chronic mucocutaneous candidiasis, autoimmune hypoparathyroidism, and Addison's disease, especially from populations such as Finns, Sardinians, and Iranian Jews should be investigated for APECED syndrome. The identification of mutations on AIRE gene is recommended to confirm the diagnosis [114].

The treatment of APECED syndrome is challenging and is mainly based on parenteral nutritional support. Immunosuppressive treatment includes high doses of intravenous steroids and methotrexate, which is well tolerated in children. Systemic chemotherapy against *Candida* infection and hormone replacement therapy are also required. Patients with APECED should be closely monitored for new components of the syndrome [122].

Prognosis

Autoimmune enteropathy is a potentially life-threatening disease. Both intestinal and extra-intestinal manifestations can lead to debilitating symptoms requiring a complex therapeutic approach. The disease itself has not been linked to the development of intestinal malignancies; however, the intensity of immunosuppressive therapy and the increasing longevity of patients potentially increase the risk of malignancy [22]. Autoimmune enteropathy is now considered as a systemic disease, and its most severe form, IPEX syndrome is often characterized by unresponsiveness to treatment with a high mortality rate [71]. Malnutrition in infants and infections in later ages are the most frequent causes of death. Given the immunologic nature of IPEX syndrome, HSCT currently seems the most promising treatment of choice. However, more experience is needed to ensure that this therapeutic approach leads to permanent remission [55, 97]. Finally, the great variability of the clinical expression and the absence of a clear genotype-phenotype correlation in APECED syndrome create the need for the identification of potential disease-modifying genes that are involved in the clinical expressivity of organ-specific autoimmunity.

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Congenital Problems of the Gastrointestinal Tract

3

Nigel J. Hall

Introduction

Congenital abnormalities of the gastrointestinal tract (GIT) are relatively common. Owing to their nature, they frequently require surgical correction, and on occasion this must be undertaken as a matter of emergency in order to avoid catastrophic intestinal ischemia and necrosis resulting in loss of bowel or damage to a secondary organ system. This chapter provides an overview of the most common conditions encountered and those which require intervention as a matter of urgency. It is not possible within the space available to describe in detail all of the variants of congenital GIT abnormalities that may be encountered and the precise nature of the treatment options available. For ease of understanding, we commence with the upper GIT and continue in a caudal direction.

Conditions Affecting the Upper Gastrointestinal Tract

Esophageal Atresia (EA) and Tracheoesophageal Fistula (TEF)

This complex group of anomalies with an incidence of 1 in 2440–4500 live births [1, 2] results from failure of correct division of the tracheal primordium from the esophagus during early embryonic development. The precise etiology is unknown with a number of embryological theories proposed to explain the different variants of this anomaly. There is a high incidence of coexisting abnormalities including the VACTERL (Vertebral column, Anorectal, Cardiac, Tracheal, Esophageal, Renal, and Limbs) syndrome, CHARGE (Coloboma of the eye, Heart defects, Atresia of the nasal choanae, Retardation of growth and/or development, Genital and/or urinary abnormalities, and Ear abnormalities and deafness) association, and isolated cardiovascular anomalies [3].

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Classification

A number of classification systems have been proposed over the years. Table 3.1 describes the most commonly encountered anatomical variants (Fig. 3.1), and the Spitz classification (Table 3.2) [3] incorporating birth weight and presence of major congenital heart disease status may be useful in predicting survival.

Clinical Features

EA is commonly associated with maternal polyhydramnios, and with increasing frequency, the diagnosis is being made antenatally particularly if there is no TEF. In the postnatal period, symptoms associated with the condition include excessive salivation, feeding difficulties, respiratory distress, and cyanotic episodes. Cases of EA (vwith the exception of the rare EA with double fistula) can be confirmed by failure of passage of a nasogastric tube into the stomach. Cases of TEF in the absence of EA (i.e., H-type fistula) may present

Table 3.1 Classification of EA/TEF anomalies and frequency

Type of lesion	Frequency (%)
EA and distal TEF	88.8
Isolated EA	7.3
H-type fistula	4.2
Distal and proximal TEF	2.8
Proximal TEF	1.1

Reprinted with permission from Hall and Pierro [4], Table 2.1, and Spitz et al. [3], Table 2 and 3, with permission from Elsevier
EA esophageal atresia, *TEF* tracheoesophageal fistula

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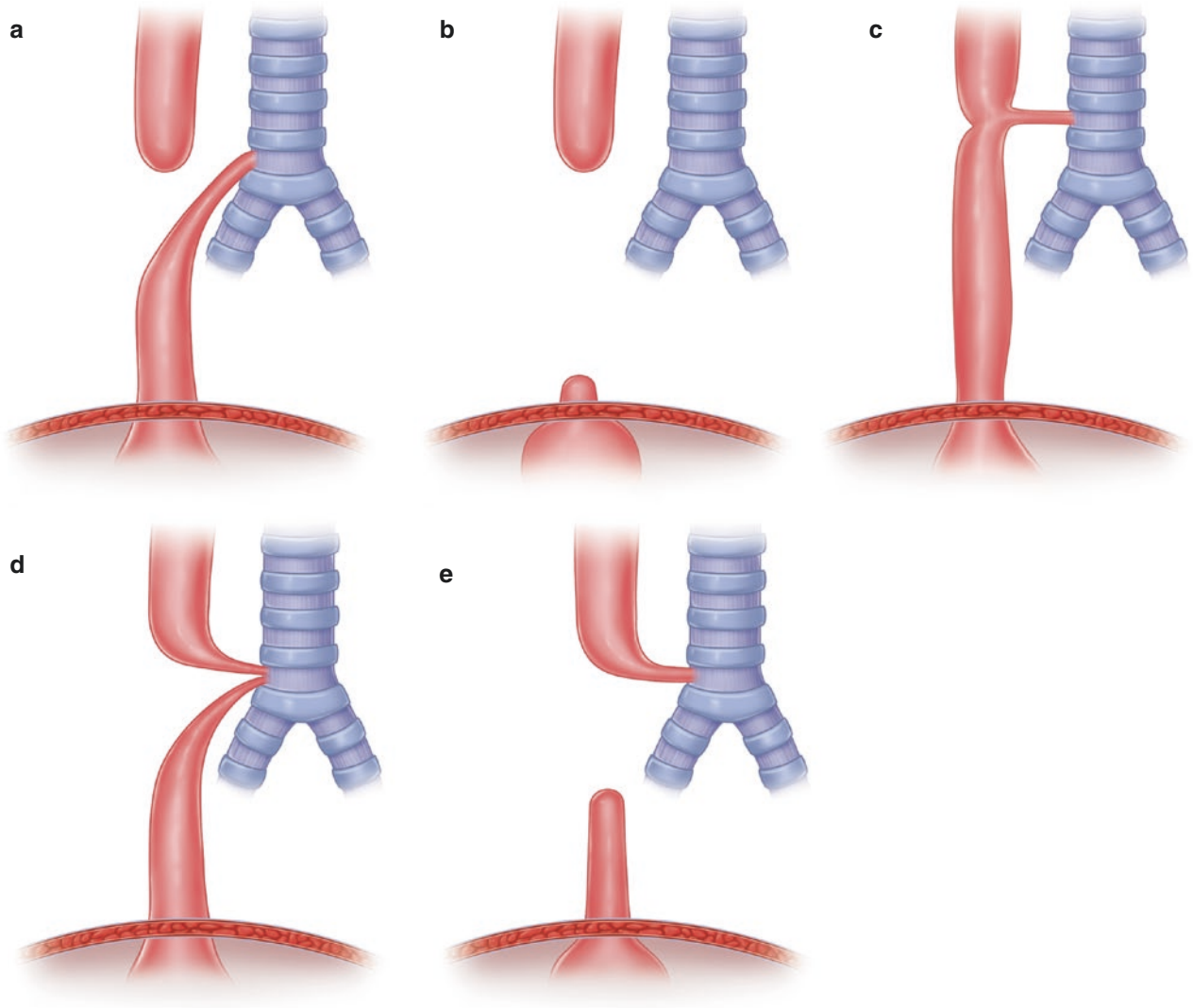

Fig. 3.1 Common anatomical variants of EA/TEF anomalies. (**a**) EA with distal TEF, (**b**) isolated EA with no TEF, (**c**) H-type TEF, (**d**) proximal and distal TEF, (**e**) EA with proximal TEF (Reprinted with permission from Hall and Pierro [4], Fig. 2.1. *EA* esophageal atresia, *TEF* tracheoesophageal fistula)

Table 3.2 Spitz classification of EA/TEF anomalies and outcome

Group	Clinical features	Survival (%)
I	BW ≥ 1500 g with no major CHD	97
II	BW < 1500 g or major CHD	59
III	BW < 1500 g and major CHD	22

Reprinted with permission from Hall and Pierro [4], Table 2.2, and Spitz et al. [3], Tables 2 and 3, with permission from Elsevier
EA esophageal atresia, *TEF* tracheoesophageal fistula, *CHD* congenital heart disease, *BW* birth weight

later, usually with recurrent episodes of respiratory distress or pneumonia.

Treatment

The overall aim of surgical correction is early division of any fistula with the respiratory tract to protect the lungs and airway, and restoration and maintenance of esophageal continuity to allow normal feeding. Following diagnosis, a Replogle tube is placed in the upper esophageal pouch which allows suction of secretions and minimizes the risk of pulmonary aspiration. Surgical repair involves ligation and division of any fistula and primary anastomosis of the two ends of the esophagus where possible. Infants who are too unstable to

tolerate primary esophageal repair, typically preterm infants with respiratory distress, may be treated with ligation of the fistula only followed by delayed esophageal anastomosis once a period of cardiorespiratory stability can be achieved. Most infants with "pure EA," that is, EA without TEF, and some infants with EA with TEF have a gap between the two ends of the esophagus that is too wide for primary anastomosis to be achieved. This group poses a particular surgical challenge. These infants are typically fed by gastrostomy initially, and the esophageal anastomosis is re-attempted after a period of growth (typically at least 6 weeks) during which the esophageal ends often grow closer together permitting anastomosis. If attempts at anastomosis remain unsuccessful, esophageal replacement such as by gastric transposition is considered.

Outcome

The majority of patients do well following anastomosis, but a number of complications may occur, and they require recurrent procedures. The most common surgical complication is anastomotic stricture. Strictures frequently require dilatation, and balloon dilatation is the preferred technique. Other complications include anastomotic leakage, recurrent TEF, gastroesophageal reflux, and disordered peristalsis. The combination of poor esophageal motility and gastroesophageal reflux with or without anastomotic stricture may lead to long-term feeding difficulties.

The Stomach

The most common abnormality of the stomach in the neonatal period is hypertrophic pyloric stenosis. Whether this is truly a congenital abnormality or an acquired disorder is questionable. It is further discussed in Chap. 4.

Other congenital conditions affecting the stomach including congenital microgastria, gastric volvulus, and congenital gastric outlet obstruction due to a pyloric web or atresia are all extremely rare and are mentioned only for completeness.

Obstructive Lesions of the Duodenum, Jejunum, and Ileum

The most common congenital conditions affecting the duodenum, jejunum, and ileum all result in partial or complete gastrointestinal obstruction. The presenting features and investigations recommended to diagnose the underlying abnormality are similar for all conditions. The clinical features and investigations of these conditions are therefore presented first followed by a description of each type of abnormality and the recommended treatment options.

Clinical Features

Obstructive lesions of the small intestine from the pylorus down to the ileocecal valve may give rise to polyhydramnios in the antenatal period which is detectable by antenatal ultrasonography. As a general rule, the more proximal the lesion, the more severe the degree of polyhydramnios, and distal ileal lesions may be present in the absence of polyhydramnios [5]. The list of differential diagnoses giving rise to polyhydramnios is however extensive. Another feature that may be seen on antenatal ultrasonography is the presence of dilated loops of intestine with or without echogenic bowel. While the combination of echogenic and dilated loops of bowel is often a sign of some form of intestinal abnormality, the precise nature of any problem is rarely identified before birth. One exception to this is a diagnosis of congenital duodenal obstruction in which case the appearance of a double bubble on antenatal ultrasonography has high sensitivity and specificity.

Following birth, the most common and important clinical manifestation of obstructive lesions of the GIT is bile-stained vomiting. Vomiting with truly bilious staining is always abnormal in the neonatal period and always requires investigation. Lesions in the duodenum and jejunum usually result in bilious vomiting within hours. In addition, the abdomen may appear empty or even scaphoid, and visible gastric peristalsis may be observed. Lesions lower in the ileum result in a distended abdomen if the obstruction is complete, and there may be failure to pass meconium. Obstructive lesions may also give rise to intestinal perforation in the neonatal period and occasionally antenatally. In all cases of neonatal intestinal obstruction, infants become progressively hypovolemic and are prone to circulatory and respiratory collapse. They require fluid resuscitation and may require ventilatory support. GIT obstruction should therefore be considered in any infant who is dehydrated especially if there is a history of vomiting.

Stenotic lesions of the small bowel in which the obstruction is incomplete may give rise to increased diagnostic difficulty. Affected infants may present with intermittent vomiting and episodes of partial obstruction. They eventually fail to thrive or develop complete obstruction at which stage they are fully investigated and the diagnosis becomes apparent.

Intestinal malrotation is considered separately as it may present with a spectrum of clinical scenarios depending on the degree of intestinal obstruction or midgut volvulus or both. The clinical pictures of all types of abnormal rotation are those of acute or chronic intestinal obstruction and/or acute or chronic abdominal pain suggestive of intestinal ischemia. True malrotation typically presents in the first year

of life with symptoms of upper GIT obstruction including vomiting which is usually bile-stained. A coexisting volvulus may be suspected by abdominal pain, peritonitis, and hypovolemic shock associated with intestinal ischemia. However, these signs may be relatively nonspecific in the young infant. Malrotation may also present later in life.

Investigations

The aim of investigating cases of suspected obstruction of the small intestine is twofold. First to identify the nature and anatomical location of the lesion to allow for planning of correct treatment and second to identify cases of malrotation in whom there is a risk of midgut volvulus and intestinal ischemia. These cases require urgent surgical intervention to reduce the risk of potentially catastrophic intestinal necrosis. The history and examination may give clues as to the location of the lesion as described above. An abdominal X-ray may simply confirm the presence of dilated intestinal loops but may also give further clues in some cases. A double bubble appearance on abdominal X-ray with a lack of air in the distal intestine (Fig. 3.2) is characteristic of duodenal obstruction. Multiple air-filled loops of proximal bowel often with air-fluid levels along with a paucity or complete absence of gas in the distal bowel is highly suggestive of obstruction of the ileum. Intestinal perforation if present will usually be apparent on abdominal X-ray, and in the rare cases of antenatal perforation, there may be widespread or localized flecks of calcification representing calcified meconium within the peritoneum.

In cases in which the diagnosis is not clear on abdominal X-ray or in which midgut malrotation or volvulus is suspected, a limited upper gastrointestinal contrast study is indicated. The classical finding in cases of malrotation is that the duodenojejunal flexure lies to the right side of the spine instead of its normal left-sided position (Fig. 3.3). This finding should prompt urgent surgical treatment due to the risk of coexisting midgut volvulus. The contrast study may also identify the presence of a stenotic segment or complete obstruction.

Cases of lower ileal stenosis or atresia are often more difficult to diagnose, and a contrast enema is invaluable in distinguishing between ileal and colonic obstruction and could be therapeutic in cases of meconium ileus (see below).

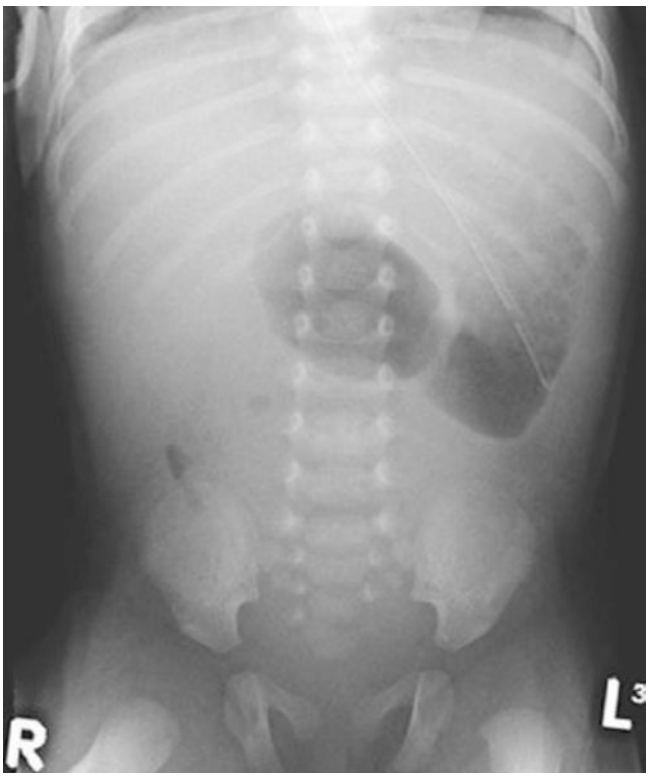

Fig. 3.2 Abdominal X-ray of an infant with duodenal atresia showing the "double-bubble" appearance characteristic of duodenal obstruction (Reprinted with permission from Hall and Pierro [4], Fig. 2.2)

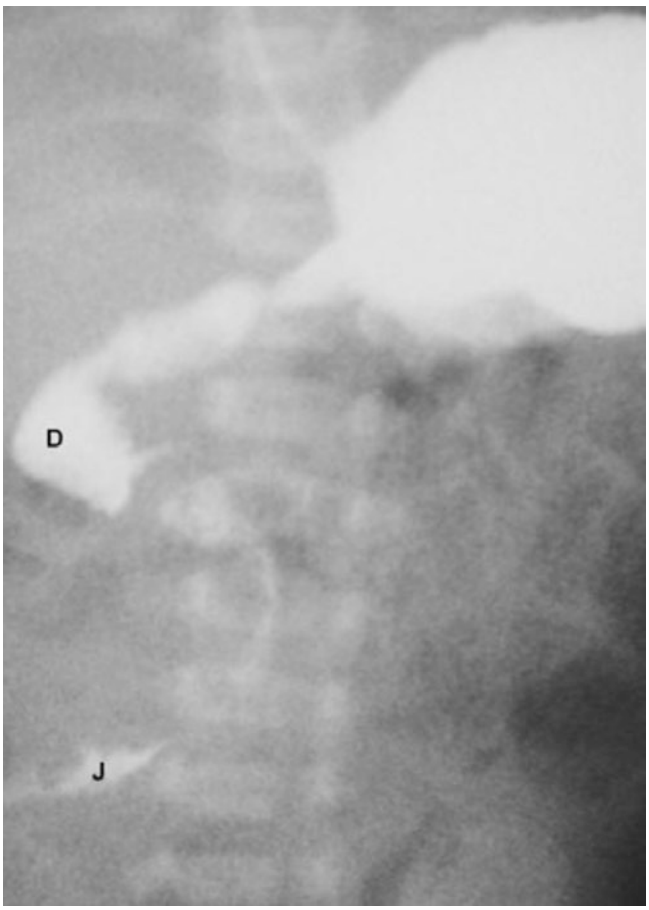

Fig. 3.3 Upper gastrointestinal contrast study of a case of malrotation. The contrast is seen within the duodenum (*D*) and flowing into the upper jejunum (*J*) both of which lie completely to the *right* of the midline (Reprinted with permission from Hall and Pierro [4], Fig. 2.3)

Conditions Affecting the Duodenum

Duodenal atresia and duodenal stenosis both of which may be associated with an annular pancreas are the commonest congenital conditions to affect the duodenum. Both are capable of giving rise to duodenal obstruction. The incidence is reported to be between 1 in 5000 and 10,000 live births [6].

Explanations of the etiology of duodenal atresias are not universally accepted. Unlike atresias of the ileum, they are not thought to be due to vascular accidents, and the most widely accepted explanation is that of failure of recanalization of the intestinal lumen during early embryonic development.

Classification

There are four basic types of duodenal obstruction (Fig. 3.4). In type 1, there is a stenosis of the duodenum resulting from a diaphragm or web partially or totally occluding the lumen. Due to the incomplete nature of the obstruction, cases may present in childhood rather than in the neonatal period. In type 2 duodenal atresia, the proximal and distal segments end blindly but remain connected by a fibrous cord. There is complete separation of the bowel segments in type 3, and type 4 comprises an atretic segment with an annular pancreas. Multiple atresias are said to occur in up to 15% of cases [7].

Treatment

The principles of treatment are to restore intestinal continuity while avoiding interference with the ductal system draining the pancreas and biliary tree. This is best achieved using a duodenostomy in which the obstructed segment is bypassed by joining the proximal segment directly to the distal segment. Following surgery, the long-term gastrointestinal results are good [8].

Conditions Affecting the Ileum and Jejunum

The main congenital problems directly affecting the small intestine from the duodenojejunal flexure down to the cecum are atresia and stenosis. Jejunoileal atresia occurs more commonly than its duodenal counterpart with an incidence of varying from 1 in 330 to 3000 live births [9]. Such lesions are one of the most common causes of neonatal intestinal obstruction. The major difference between atresias of the ileojejunum and those of the duodenum is in their etiology. It is postulated that atresia or stenosis of the jejunum and ileum is the result of a localized vascular accident during intrauterine life. Subsequent ischemic necrosis and reabsorption of the affected segment or segments result in a contracted scarred bowel wall leading to stenosis at one end of the spectrum to a complete intestinal and mesenteric defect at the other. Fetal animal experiments have confirmed at least in part this hypothesis [10], and the absence of other congenital abnormalities found in association with jejunoileal stenoses and atresias supports the localized vascular accident theory.

Classification

Morphological classification of these lesions allows different surgeons and centers to compare outcomes, and it is also of therapeutic and prognostic value. The most commonly accepted system is that proposed by Louw [11] and modified by Grosfeld [12]. Whether the lesion is classified as ileal or jejunal is determined by the most proximal affected segment (Fig. 3.5).

Stenosis is a localized narrowing of the lumen without any break in the continuity or mesenteric defect. The intestinal wall may be thickened and rigid at the stenotic site, and there is a small, often minute lumen. The overall intestinal length is not shortened. Type I atresia is the result of a membranous web occluding the lumen with no mesenteric defect and no intestinal shortening. The lumen is usually completely occluded, and the proximal bowel therefore dilated but remaining in continuity with the collapsed distal segment. Type II atresia arises from a complete obliteration of the intestinal segment into a fibrous cord which joins two blind ends and runs in the free edge of the mesentery. There is no mesenteric defect, and once again, the total bowel length is usually normal. In both type III(a) and III(b) atresia, the intestine is likely to be shortened, and this may have significant clinical consequences. Type III(a) atresia consists of blind-ending proximal and distal bowel with no connection and an often large mesenteric defect. The blind ends are often physiologically abnormal with decreased or absent peristaltic activity which may give rise to torsion, distension, or perforation. Type III(b) atresia, also known as apple-peel atresia because of its gross morphology, may involve massive intestinal loss. It consists of intestinal atresia near the ligament of Treitz, obliteration of the superior mesenteric artery (SMA) beyond the origin of the middle colic branch, and the absence of the dorsal mesentery. The remaining intestine is coiled helically (like an apple peel) around a single perfusing vessel, often has impaired vascularity, and is almost inevitably short. Furthermore, there may be additional segments of type I or II atresia within the apple-peel segment. Such a configuration most likely arises from occlusion of the SMA due to thrombus,

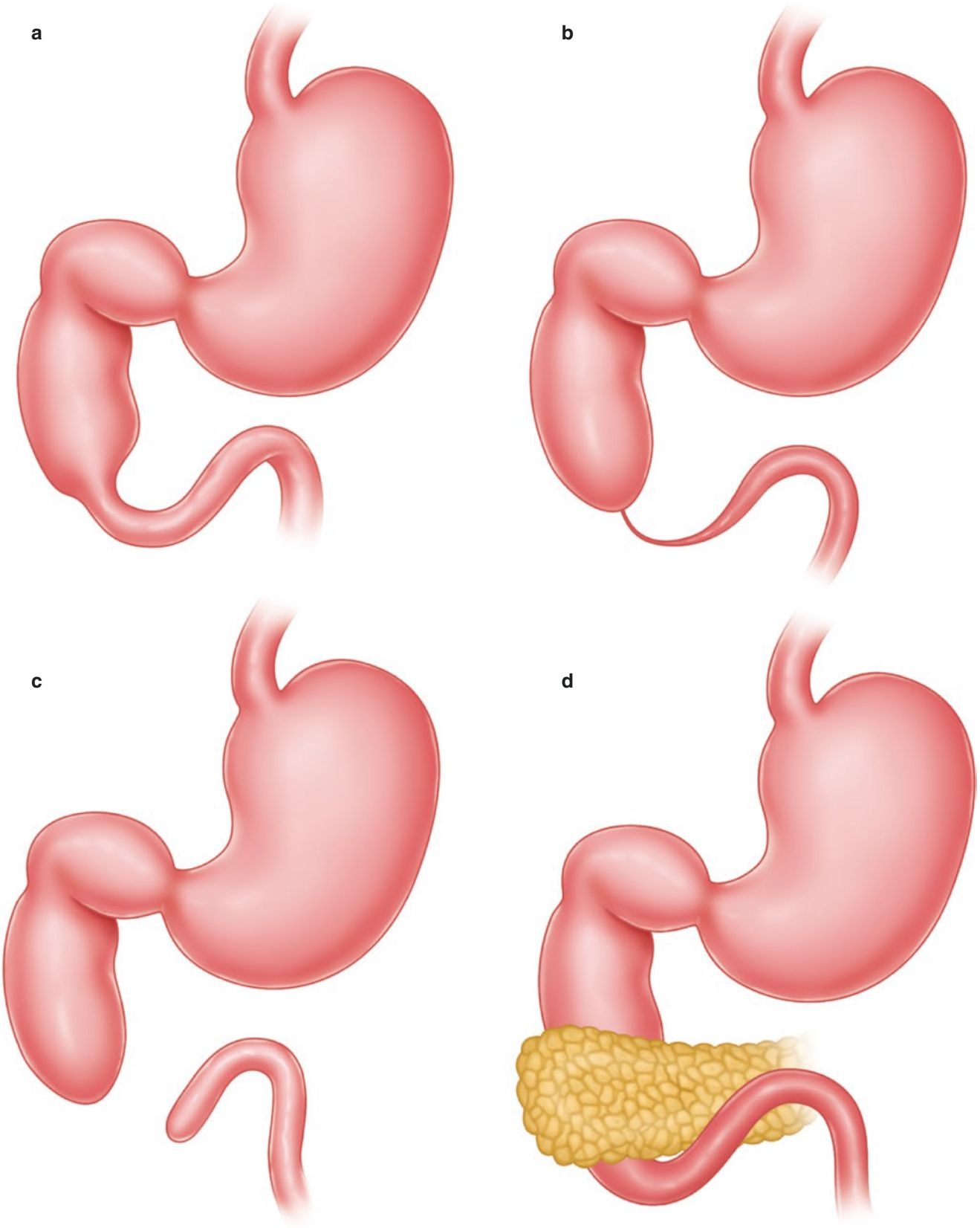

Fig. 3.4 Variants of duodenal atresia. (**a**) Type 1 atresia due to an internal diaphragm, (**b**) type 2 atresia with blind-ending loops remaining connected by a fibrous cord, (**c**) type 3 atresia with blind ends completely separated, (**d**) type 4 duodenal obstruction with an annular pancreas (Reprinted with permission from Hall and Pierro [4], Fig. 2.4)

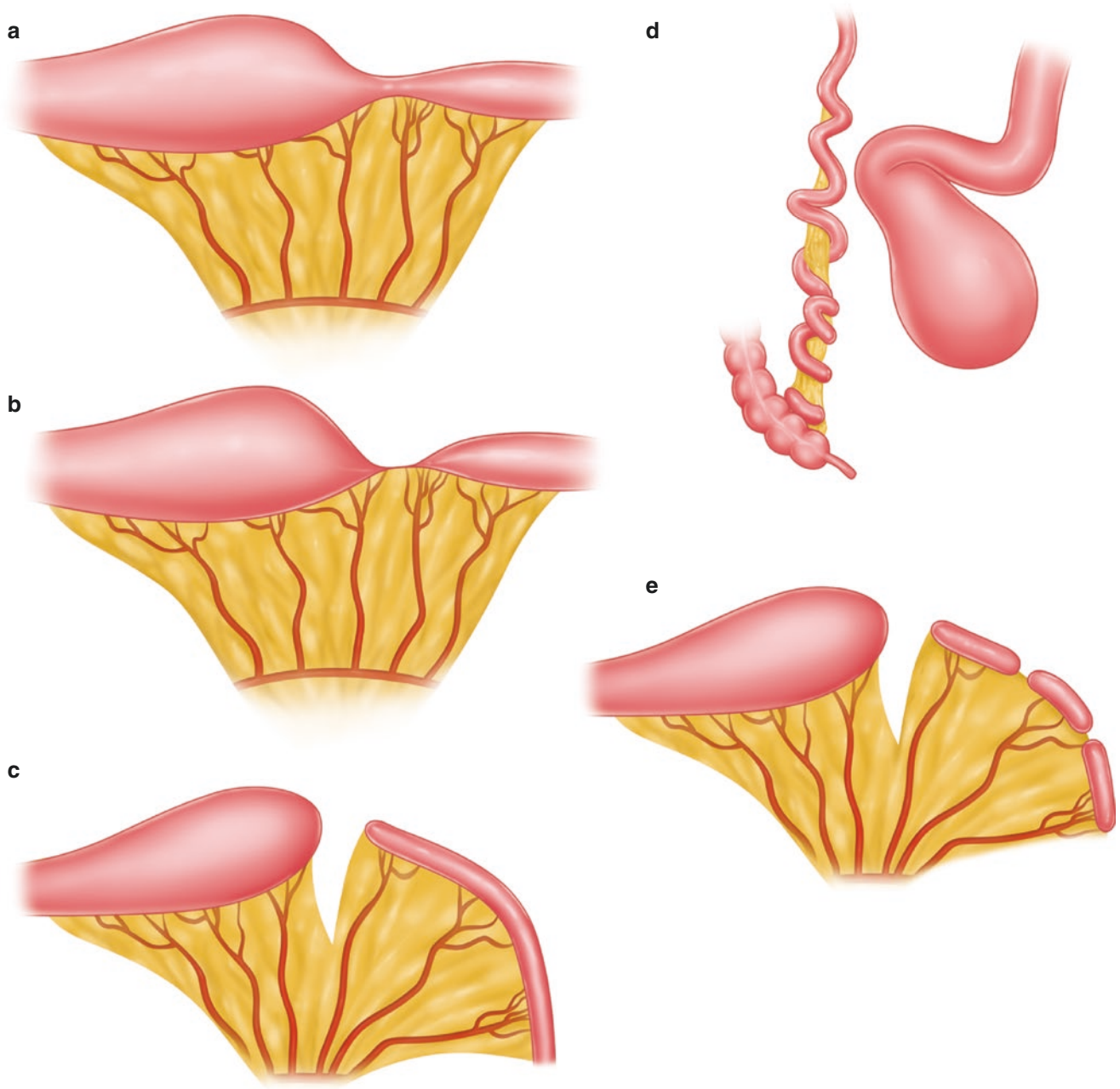

Fig. 3.5 Variants of ileal atresia. (**a**) Type I due to an internal web (not shown) with no mesenteric defect, (**b**) type II atresia with blind ends joined by a fibrous cord, (**c**) type III(a)—blind ends separated with a mesenteric defect, (**d**) type III(b) in which the ileum is coiled like an "apple peel" around a single vessel and completely separated from the proximal dilated jejunum, (**e**) type IV or multiple atresias (Reprinted with permission from Hall and Pierro [4], Fig. 2.5)

embolus, or strangulation as part of a midgut volvulus. In type IV atresia, there are multiple atretic segments, and the intestine may resemble a string of sausages. Overall, bowel length is usually shortened, and the intestine grossly dilated. It has been proposed that the etiology of type IV atresia may be due to failure of recanalization of solid epithelialization throughout the length of the intestine rather than from multiple single vascular events.

While it is generally accepted that stenosis and atresia of types I, II, and III(b) are the result of intrauterine vascular accidents, a genetic component has been suggested in type III(b) and IV.

Treatment

The mainstay of surgical treatment for this type of lesion is resection of the atretic or stenotic segment and primary anastomosis with closure of the mesenteric defect. The proximal intestinal segment is usually dilated and functionally abnormal with absent or ineffective peristalsis. This dilated proximal segment is excised along with a short segment distal to the stenosis or atresia. It is essential to establish patency of the distal bowel by irrigation or wash out intestine, and subsequently, a primary anastomosis is performed. There is a balance to be struck between the length of dilated proximal segment resected and the risk of leaving the infant with a short length of small bowel. As such, it is almost inevitable that the caliber of proximal bowel will be greater than that of the distal intestine, and a number of techniques exist to assist construction of the anastomosis in such circumstances.

Outcome following intestinal atresia is dependent primarily on the length of remaining intestine and the presence of the ileocecal valve. Short bowel syndrome has been defined as the presence of less than 75 cm of the small intestine or 30% of the predicted intestinal length in a premature infant [13, 14]. Outcomes following short bowel syndrome vary, and there is a high level of dependence on parenteral nutrition. However, intestinal adaptation can occur such that more than 80% of babies with short bowel syndrome do eventually become entirely enterally nourished [15].

Intestinal Malrotation

The incidence of intestinal malrotation is difficult to truly establish as not all affected patients develop symptoms, but autopsy studies estimate the incidence at approximately 1 in 500.

The traditional embryological basis for disorders of intestinal rotation is that of abnormal positioning of the intestinal loops in relation to one another as they return to the abdominal cavity from the yolk sac. During normal development, the midgut rotates through 270° so that the duodenum lies posterior to the colon, and the duodenojejunal flexure is to the left of the midline. A consistent finding in cases of malrotation is abnormal positioning of the duodenojejunal flexure. However, an alternative hypothesis has been proposed based on animal studies [16]. Kluth proposes that malrotation is the result of failure of localized growth of the duodenal loop rather than a disorder of rotation.

The term "malrotation" covers a spectrum of anatomical abnormalities. In non-rotation, the duodenojejunal flexure lies to the right of the spine along with most of the small intestine. The cecum and colon are typically on the left side of the abdominal cavity. Adhesions formed between loops of bowel or the intestine and abdominal wall are usually responsible for obstructive symptoms at presentation. In malrotation, the distribution of intestinal contents within the abdominal cavity is such that the duodenum again lies to the right of the spine with the cecum anterior to it. Adhesions between these two structures (Ladd's bands) are often present and may result in partial or total occlusion of the second part of the duodenum. In addition, the mesenteric attachment to the posterior aspect of the abdominal cavity is typically very short, and there is a risk of volvulus with ensuing intestinal ischemia. Other forms of abnormal intestinal rotation (inversed rotation, malrotation with mesocolic hernia, and malposition of the cecum) are all rare.

Treatment

There are two aspects to this disorder which require surgical intervention. The first and most important aspect is that of midgut volvulus. Any infant in whom malrotation is suspected based on clinical findings and radiological investigations should undergo laparotomy as a matter of urgency in order to minimize the risk of intestinal ischemia due to a volvulus. At laparotomy, blood-stained peritoneal fluid may indicate the presence of ischemic intestine. Any volvulus should be derotated (usually in clockwise direction), and the intestine examined for viability. Nonviable bowel is resected, and a primary anastomosis performed. If there is doubt about the viability of remaining intestine, a second-look laparotomy can be performed after 24 h.

In cases of malrotation not complicated by volvulus, the procedure of choice for most surgeons is the Ladd's procedure. This involves division of all adhesions or adhesive bands between the cecum, duodenum, and parietal peritoneum, broadening of the mesenteric base around the SMA and repositioning of the intestine within the abdominal cavity such that the duodenum is on the right and the cecum lies in the left upper quadrant. Some surgeons recommend performing an appendicectomy due to the difficulties of diagnosis should appendicitis develop later in life.

Meconium Ileus

Meconium ileus is a common cause of neonatal intestinal obstruction and the commonest cause of antenatal intestinal perforation [17]. It should be included in the differential diagnosis of infants presenting with GIT obstruction. In approximately 80% of cases, it is associated with cystic fibrosis [18–20], an autosomal recessive disease affecting predominantly the lungs and pancreas. The underlying defect in cystic fibrosis, an abnormality in a transmembrane chlo-

ride channel, results in the production of abnormally viscid and sticky meconium. This meconium sticks to the intestinal mucosa causing intestinal obstruction usually occurring late in gestation. Why some infants with cystic fibrosis do not develop meconium ileus is unclear. Meconium ileus can be classified as: (1) "uncomplicated" when is limited to intraluminal obstruction caused by the abnormal meconium or (2) "complicated" when is associated with intestinal atresia, volvulus, or meconium peritonitis.

Clinical Features

In cases of uncomplicated meconium ileus, the infant usually presents shortly after birth with symptoms of lower gastrointestinal obstruction, including abdominal distension and vomiting which may or may not be bile stained. The rectum may appear empty and narrow on plain radiograph, and the infant does not pass meconium. If meconium ileus is complicated by volvulus, intestinal ischemia, or perforation, the infant can be systemically unwell with acidosis and hypovolemic shock and may require ventilatory support. Abdominal X-ray showing dilated intestinal loops and occasionally abundance of meconium in the right lower quadrant are supportive of the diagnosis as is a gastrografin contrast enema revealing a small collapsed colon (microcolon) and often inspissated pellets of meconium in the right lower quadrant.

Treatment

In some cases, the gastrografin enema mentioned above may relieve the obstruction sufficiently to be curative. However, a number of uncomplicated cases and all complicated cases require surgery. The procedure performed depends on the findings during laparotomy. Atretic or grossly dilated segments of bowel may be resected, the inspissated meconium removed from the intestinal lumen, and the distal bowel flushed through. Occasionally, a stoma is formed to allow intestinal decompression. Outcome of surgical treatment is generally good, and gastrointestinal complications are of lesser significance than the pulmonary disease caused by the underlying cystic fibrosis.

Meckel's Diverticulum

Meckel's diverticulum is the commonest omphalomesenteric remnant with a reported incidence of approximately 2%. Of these, only a small proportion become clinically significant. The diverticulum originates from incomplete obliteration of the omphalomesenteric duct and exists as a free-lying diverticulum on the anti-mesenteric border of the ileum.

Clinical Features

There are a variety of disease entities attributed to a Meckel's diverticulum including gastrointestinal hemorrhage, intussusception, diverticulitis, and perforation. The commonest presenting symptom is that of gastrointestinal bleeding due to excessive acid and pepsin production from ectopic gastric mucosa which may be present within the diverticulum. Bloody diarrhea in the absence of abdominal pain is the classical presenting picture. Other complications of Meckel's diverticulum are intussusception in which the diverticulum acts as a lead point, diverticulitis with symptoms similar to those of appendicitis, and perforation.

Treatment

Management of all clinically significant cases of Meckel's diverticulum is resection of the diverticulum after adequate preoperative resuscitation. At operation, the diverticulum and a wedge of ileum are resected. The ileal wedge is included as ectopic tissue may not be entirely confined to the diverticulum. Following surgical excision outcome is good.

Congenital Hepatic, Pancreatic, and Biliary Abnormalities

Abnormalities of the hepato-pancreato-biliary system are all quite rare. They are included here as knowledge of their existence is important as they form part of the differential diagnosis for infants with jaundice, malabsorption, and hypoglycemia.

The commonest lesions of the biliary tree are biliary atresia and congenital biliary dilatation. In biliary atresia, the biliary tree is obliterated either completely or partially. Congenital biliary dilatation describes a variety of abnormalities of the biliary tree in which the dilated segment may be either intrahepatic or extrahepatic and either fusiform or cystic in nature. Dilatations of the extrahepatic biliary ducts are commonly known as choledochal cysts. The dilated bile duct is both anatomically and functionally abnormal resulting in cholestasis. Infants with biliary atresia and severe cholestasis associated with biliary dilatation present in the neonatal period with prolonged jaundice due to accumulation of conjugated bilirubin. When the degree of obstruction to the biliary tree is not so severe, congenital biliary dilatation may present later in life with malabsorption, intermittent jaundice, abdominal pain, or even pancreatitis. In addition, a choledochal cyst may present as an upper abdominal mass. Treatment of these lesions is based on allowing drainage of the biliary tree into the intestine, and the surgery involved is often complex. The operation of choice for biliary atresia is

the Kasai porto-enterostomy [21]. The atretic remnants of the extrahepatic biliary ducts are removed, and the porta hepatis is anastomosed to a defunctioned loop of jejunum. The timing of surgery is of paramount importance to avoid hepatocellular damage, but even with prompt diagnosis and early surgical intervention, infants with biliary atresia often have residual hepatic impairment due to intrauterine cholestasis. In cases of choledochal cysts, the dilated portion of the extrahepatic ducts is removed together with the gallbladder, and the common hepatic duct is anastomosed to the duodenum or a defunctioned loop of jejunum.

There are a number of congenital hepatic anomalies which give rise to structural and/or functional abnormalities of the liver parenchyma or the intrahepatic biliary tree. These include infantile and adult-type polycystic disease, congenital hepatic fibrosis, biliary hypoplasia, and congenital tumors of the liver such as hamartomas and hemangiomas. Presentation is usually with one of hepatomegaly, portal hypertension, or cholangitis. Treatment is that of resection of suitable lesions and prevention or treatment of hepatic disease.

Congenital lesions involving the pancreas are rare, the commonest being annular pancreas (see section on the duodenum). Other anatomical anomalies are seen including pancreatic ductal anomalies, pancreatic cysts, and very rarely pancreatic agenesis. There are a group of infants who present in the neonatal period with hypoglycemia who are found to have inappropriately high levels of circulating insulin. The condition hyperinsulinemic hypoglycemia (previously commonly referred to as "nesidioblastosis") is characterized by inappropriate endogenous insulin secretion in the presence of low blood glucose. It may result from an insulin-secreting tumor in the pancreas (a so-called insulinoma), but more commonly, no tumor is identified and the disease is a result of a genetic defect in a membrane channel controlling insulin secretion. Infants require high glucose intake to maintain normoglycemia while investigated for the presence of an isolated secretory tumor. Treatment is by surgical excision of the tumor if present; otherwise, a 90–95% subtotal pancreatectomy is performed.

Conditions Affecting the Lower Gastrointestinal Tract

Hirschsprung Disease

Hirschsprung disease is the commonest congenital malformation of the enteric nervous system with an incidence of approximately 1 in 5000 live births [22–24]. While most cases are sporadic, a positive familial occurrence exists in 3.6–7.8% of cases [25], and the presence of coexisting abnormalities including trisomy 21 suggests a genetic involvement. The condition is fully addressed elsewhere (see Chap. 22).

Anorectal Anomalies

Congenital abnormalities of the anorectal region occur with an incidence of 1 in 4000–5000 live births [26–28]. A very small minority may be familial with the majority being isolated findings or part of a congenital syndrome such as the VACTERL syndrome. There are a number of different types of anorectal anomalies resulting from the complex embryological development of the anorectal region involving differentiation of the cloaca. The Wingspread classification [29] divides them into high, intermediate, or low based on the relationship of the terminal bowel or any fistula arising from the bowel to the pelvic diaphragm. Precise definition of the abnormal anatomy is of paramount importance when planning corrective surgical treatment.

Clinical Features

Abnormalities of the anorectal region are usually diagnosed on inspection during the newborn period, but surprisingly, this is not always the case. In many cases, the anus will be absent, and meconium may be seen to originate from an abnormal site including a mucocutaneous fistula, the urethra in males, or the vaginal vestibule in females. Anomalies in which the anus is present but abnormally sited or stenosed may be more difficult to diagnose in the absence of adequate experience. The most complex abnormality in females is represented by the cloaca which is represented by a single opening in the perineum with rectum, vagina, and urethra joining a single channel.

Treatment

Treatment is aimed at preventing complications associated with the anomaly including urinary tract infection and lower gastrointestinal obstruction and subsequently restoring the anatomy to as near to normal functional and cosmetic state as possible. In the majority of cases, the initial surgery involves forming a colostomy in the descending or sigmoid colon to allow intestinal drainage and avoid dilatation of the lower bowel [30]. Following assessment, planning of surgery and growth of the infant reconstructive surgery is undertaken most commonly by the posterior sagittal approach [31]. Some anomalies also require a laparotomy to divide a high recto-vesical fistula. Surgery of these cases and particularly

of cloaca is complex and should be performed by an experienced surgeon.

Outcome

In similarity to patients with Hirschsprung disease, incontinence and constipation are the most significant long-term complications of anorectal anomalies and often have a significant impact on quality of life. In one large series, soiling occurred in 57% of 387 cases. The incidence of fecal incontinence was 25% and constipation 43% [31]. Ongoing medical and on occasion surgical treatment is necessary to minimize disruption to a normal lifestyle.

Conditions Which May Occur at Any Point in the Gastrointestinal Tract

Gastrointestinal Duplications

Duplication cysts of the GIT are rare congenital abnormalities. They can occur at any point in the GIT from mouth to anus, although they are most commonly found around the ileocecal region. Duplication cysts are defined according to strict criteria as devised by Ladd and Gross; they are closely attached to some part of the GIT, have a smooth muscle coat, and have an epithelial lining which resembles some part of the alimentary canal [32]. Duplications may be spherical or tubular in macroscopic appearance, those that are tubular accounting for 10–20% and often having a communication with the bowel.

Clinical Features

Between 25% and 30% present in the neonatal period and most have presented by the age of 10 years. Clinical features at presentation depend on anatomical site, size, and secondary effects and include an oropharyngeal, abdominal or rectal mass, respiratory distress, gastrointestinal bleeding, obstruction, and intussusception. Duplication cysts may also be found as incidental findings at laparotomy, and some lesions have been detected on antenatal ultrasound [33, 34].

Treatment

The recommended management of duplication cysts is complete surgical excision wherever possible in order to prevent recurrence and complications secondary to ectopic gastric mucosa. When complete excision is not possible, it is essential to remove the mucosal lining.

Conditions Affecting the Walls of the Abdominal Cavity

While not truly conditions of the GIT, there are a number of conditions which cause the abdominal contents to develop outside the abdominal cavity. These conditions are included as they have secondary effects which may significantly affect the GIT and be a cause of gastrointestinal dysfunction.

Congenital Diaphragmatic Hernia

The incidence of congenital diaphragmatic hernia (CDH) varies from 1 in 3500 to 1 in 5000 live births [35]. Its etiology is unknown although is probably multifactorial. The essential anatomical defect is a breach in the continuity of the diaphragm which allows herniation of the abdominal viscera into the thoracic cavity. This has a secondary effect of impeding development of the lungs during intrauterine life. The resulting hypoplastic lungs are a cause of significant morbidity and mortality in this condition. Compression by misplaced abdominal contents does not explain the severity of lung disease seen, and it is well recognized that lung development is markedly abnormal in infants with CDH.

Classification

A number of different defects can occur owing to the complex development of the diaphragm. The majority of cases are of the Bochdalek type in which the defect is posterolateral and most commonly on the left side. The defect can range in size from a small slit to involve almost the entire hemidiaphragm. Defects in the central tendon of the diaphragm result in Morgagni hernias which are retrosternal in nature and most commonly on the right side. Finally, agenesis of the diaphragm may occur which is usually left sided and extremely rare.

Clinical Features

In the current era, diaphragmatic hernia is often diagnosed during prenatal ultrasound scanning. The advantage of prenatal diagnosis is that delivery can be planned to take place in a unit with appropriate pediatric surgical and intensive care facilities. For those infants who avoid prenatal diagnosis for whatever reason, the clinical features depend on the volume of abdominal contents within the thoracic cavity and the degree of lung hypoplasia. In the most severe cases, there will be severe respiratory distress and cyanosis from shortly after birth. At the other end of the spectrum are infants who

have minimal if any respiratory symptoms or signs and in whom intestinal loops are noted to be in the abdomen on chest X-ray (Fig. 3.6).

Treatment

While the definitive treatment of diaphragmatic hernia is surgical closure, the timing of this is not of paramount importance and should be undertaken once the infant is stable from a cardiovascular and respiratory point of view. The respiratory management of these infants can be problematic due to the severe lung hypoplasia and associated pulmonary hypertension. Most require conventional ventilatory support as a minimum, and many require high-frequency oscillatory ventilation (HFOV) to ensure adequate oxygenation. Other measures to reduce pulmonary hypertension including inhaled nitric oxide, adenosine, or sildenafil may be effective. Resistant cases may be candidates for extracorporeal membrane oxygenation (ECMO), during which the infant is placed on a life support system in the hope that the lungs and in particular the pulmonary vasculature will mature. Various criteria for ECMO exist [36] with the aim of reserving it for those who have the most severe respiratory failure and those who are most likely to benefit. There remain unfortunately a number of infants with CDH whose lung disease presents too great a challenge, and they do not survive.

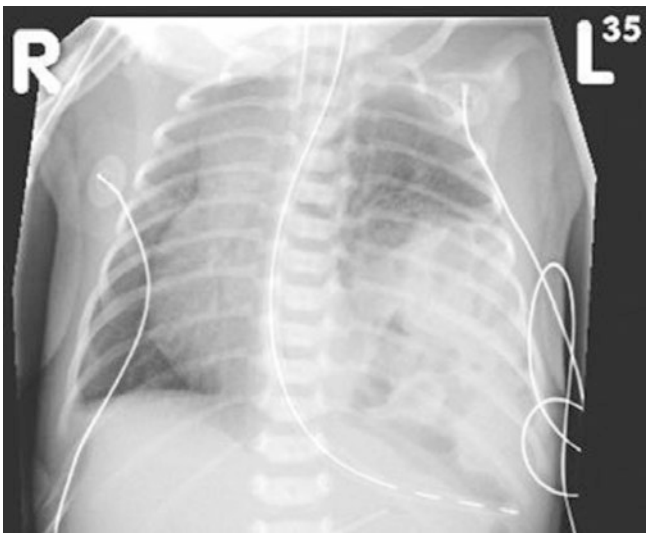

Fig. 3.6 Chest radiograph of an infant with congenital diaphragmatic hernia. Loops of intestine are clearly seen within the *left* hemithorax, and there is mediastinal shift to the *right* (Reprinted with permission from Hall and Pierro [4], Fig. 2.7)

Surgery

The principles of surgical repair are to return the abdominal contents to the abdominal cavity and repair the diaphragmatic defect. It may be possible to repair the defect by simply suturing the edges together. However, if the defect is large, a patch repair may be undertaken using prosthetic material. The long-term outcome of CDH is dependent primarily on the degree of pulmonary hypoplasia. The main gastrointestinal consequence appears to be gastroesophageal reflux seen in up to 62% of cases [37].

Anterior Abdominal Wall Defects

Although separate clinical entities, the two conditions exomphalos and gastroschisis which comprise anterior abdominal wall, defects are grouped together due to similarity in their clinical appearance and the recommended course of management. In both conditions, some portion of the viscera lies outside the abdominal cavity extruding through a defect in the anterior abdominal wall.

Exomphalos

The defect on the anterior abdominal wall in cases of exomphalos lies in the midline. Viscera herniate through this defect but remain contained within an avascular hernial sac consisted of peritoneum and amniotic membrane (Fig. 3.7).

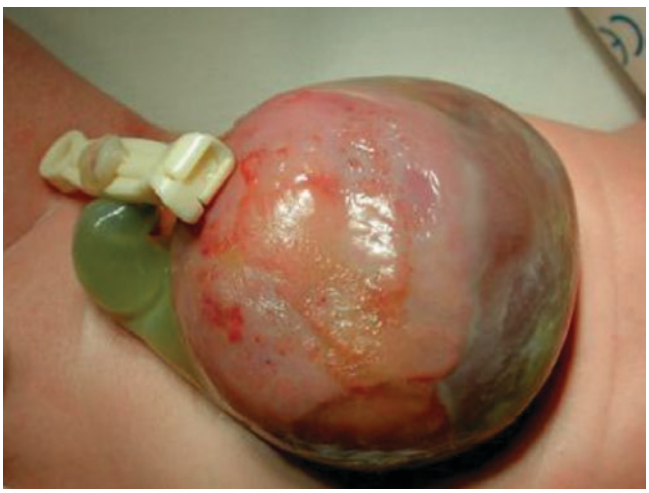

Fig. 3.7 Clinical appearance of an infant with exomphalos. The abdominal contents are enclosed within an avascular hernial sac (Reprinted with permission from Hall and Pierro [4], Fig. 2.8)

The size of the defect and hence the size of the sac may vary in size from a small swelling at the base of the umbilical cord (exomphalos minor) to a much larger sac containing liver and a large proportion of the small intestine (exomphalos major). The embryological origins of exomphalos are believed to be failure of complete closure of the anterior abdominal wall around a persistent body stalk. Visceral contents continue to develop within this body stalk and thus remain outside the abdominal cavity. While the precise etiology of exomphalos is not clear, it is well recognized that exomphalos often coexists with a number of other congenital abnormalities, and this may suggest at least in part a genetic component. Associated abnormalities include Beckwith–Wiedemann syndrome; the trisomies 13, 18, and 21; and the upper and lower midline associations.

Gastroschisis

The anterior abdominal wall defect in cases of gastroschisis is full thickness and typically to the right of the umbilical cord. Unlike exomphalos, there is no sac covering the eviscerated intestine which is usually dilated and inflamed (Fig. 3.8). The liver is not herniated. The precise embryological basis of gastroschisis is unclear, and a number of hypotheses have been proposed. The fact that gastroschisis is rarely associated with any other congenital abnormalities—with the exception of intestinal atresias and malrotation—suggests that the events resulting in exomphalos are likely to have a separate embryological basis.

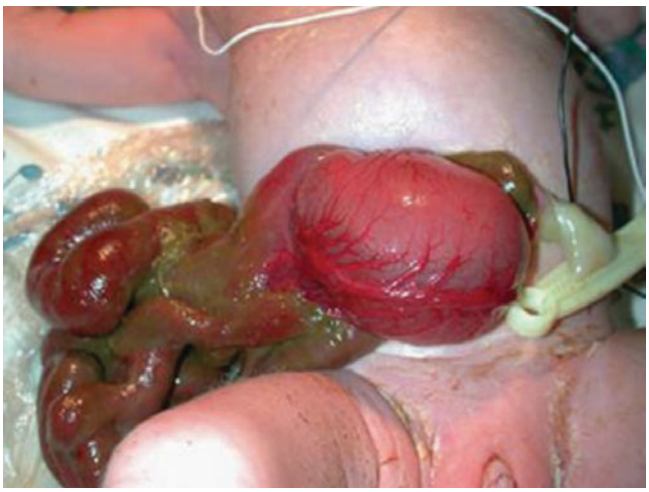

Fig. 3.8 Clinical appearance of an infant with gastroschisis. There is no sac enclosing the herniated intestine which is thickened and inflamed (Reprinted with permission from Hall and Pierro [4], Fig. 2.9)

Treatment

It is now usual for these two abnormalities to be detected in the antenatal period, and delivery in a specialist center with pediatric surgical facilities is recommended. In gastroschisis, there is no convincing evidence to suggest that preterm or caesarean section delivery confers any distinct advantage [38–40], but delivery is commonly induced at around 37 weeks gestation to avoid late gestation fetal death [41]. What is of paramount importance is protection of the intestine and prevention of fluid loss in cases of gastroschisis from the moment of delivery. The eviscerated intestine is wrapped in clingfilm and adequate support provided to prevent fluid loss and ischemic damage to the bowel. For exompahalos vaginal delivery is possible except in cases of exomphalos major. The hernial sac in exomphalos provides protection to the underlying bowel and surgical intervention is usually not emergent. However, cases of exomphalos in which the hernial sac ruptures during delivery should subsequently be treated as for gastroschisis.

Surgical Closure

The aim of surgery in both conditions is the return of abdominal contents to the abdominal cavity and closure of the overlying skin. In some cases, this can be achieved in one surgical procedure (primary closure), but in many instances, the abdominal cavity is not of sufficient volume to accommodate the eviscerated organs. In these cases, a staged closure is performed in which a "silo" is attached under the fascia around the base of the defect and completely encloses the eviscerated abdominal contents. This gives the intestine protection from dehydration and contains the bowel within a manageable sac reducing the risk of intestinal damage. Recently some surgeons have been preferentially using a silo for all cases of gastroschisis at least in part due to the belief that a more gradual return of the bowel to the abdomen may result in favorable outcomes [41–44]. The silo is gradually reduced in size as the abdominal cavity allows and the skin closed either in a final operation or using a technique of non-operative closure. In cases of exomphalos major, some surgeons elect not to reduce the viscera to the abdominal cavity in the newborn period but to allow the sac to epitheliase and perform fascial repair during childhood. Following abdominal wall closure, there is a period of intestinal dysmotility which is much more pronounced in infants with gastroschisis. During this period nutrition is maintained using parenteral nutrition. The overall prognosis for infants with both these conditions in the absence of coexisting abnormalities is good [45].

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Pyloric Stenosis

4

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Introduction

Pyloric stenosis is a common pediatric surgical condition. It involves both narrowing and lengthening of the pylorus due to hypertrophy of the pylorus muscle. As this diagnosis is most often found in the first few months of life, it is often referred to as infantile hypertrophic pyloric stenosis (IHPS).

Hirschsprung first clinically described IHPS in 1888; however, it was much earlier, in 1717, that Blair actually reported findings of pyloric stenosis following an autopsy of a 5-month-old infant [1]. In 1911, Conrad Ramstedt incidentally developed pyloromyotomy, which has remained the standard procedure for the treatment for more than a century. For long time it was known as Fredet-Ramsted pyloromyotomy, Pierre Fredet being credited for extramucosal pyloroplasty, which was later modified by Ramsted.

Incidence

Incidence of IHPS is approximately 4 in 1000 live births, with a higher prevalence among the Caucasian population. Males appear to be affected much more commonly than females, at a 4:1 ratio. Child presents between age of 3 and 12 weeks [2] and is rarely seen in babies over 6 months of age. Several recent European studies and a nested case-control study from Israel have suggested a consistent decrease in IHSP incidence, which is most likely attributed to changes in environmental factors [3]. Condition is more commonly seen in children whose mother had pyloric stenosis compared to the one whose father has PS.

Anatomy

The pylorus is a muscle, which forms an anatomical connection between the stomach and the duodenum. It consists of two parts: the pyloric antrum and the pyloric canal, which connect to the body of the stomach and the initial part of the duodenum, respectively, allowing the passage of nutrients from the stomach to the small intestine, aiding the digestive process. Before the pyloric antrum, a prepyloric sphincter is positioned, which can constrict allowing the stomach to be shut as a separate entity from the small intestine for brief seconds during peristaltic waves. Additionally, at the end of the distalpyloric canal, a pyloric sphincter is located, which is fundamental to allow food passage from the stomach to the small intestine. Pylorus hence acts as a muscular valve preventing food from re-entering the stomach once it has passed into the small intestine.

The pyloric sphincter consists of circular smooth muscle, allowing the sphincters to contract and relax as required. As the content of the duodenum increases, this causes an increase in pressure, closing the pyloric sphincter, thus controlling the amount of food that is digested at any one time. A mucosal membrane underlines this sphincter, allowing gastric secretions to enter the pylorus further aiding digestion. As a general rule, the pylorus mainly refers to the pyloric sphincter, and therefore IHPS refers to a narrowing of the sphincter due to hypertrophy of the circular muscle. As hypertrophy occurs, this also causes the pyloric canal to lengthen and leads to a gastric outlet obstruction.

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Histology

Under normal conditions, the pylorus, when viewed under a microscope, contains a number of features that allow for successful digestion. These include gastric pits and parietal cells, secreting gastric acid, as well as neuroendocrine cells to allow communication between the brain and gastrointestinal tract, enabling efficient contraction and secretion. Furthermore, Auerbach's plexus is implicated to play an important role in gut motility, by providing sympathetic and parasympathetic innervation to the muscular layers of the gut. However, during IHPS, it has been found that there is a degeneration of intramuscular ganglion cells in Auerbach's plexus, along with the accretion of lysosomes and cytoplasmic bodies in many of the axons [2]. Additionally, the ganglion cells appear smaller and reduced in number [4]. Cameron [5] has suggested that due to both the degeneration and consequent reduction in ganglia cells, Auerbach's plexus is ineffective, causing a reduction in neural activity, leading to asynchronous contraction of the pyloric muscle. As contractions are asynchronous, they increase in an attempt to produce a successful contraction, which is thought to lead to hypertrophy of the circular muscle, and hence IHPS.

Etiology and Risk Factors

Etiopathogenesis of pyloric stenosis is still not clearly understood. It is thought to be multifactorial with both environmental and genetic factors contributing toward the disease manifestation.

Genetic Factors

There appears to be a familial link for pyloric stenosis, but the specific genetic factors involved have not yet been identified. In a study conducted in Denmark in 2010, 1,999,738 children were studied in their first year of life, with findings of 3362 having IHPS. From the study, it was identified that, for a singleton, the incidence rate for IHPS was 1.8/1000, compared to 3.1/1000 live births in twins. Additionally, the rate ratio was 182 for monozygotic twins, compared to only 29.4 for dizygotic twins, illustrating a strong genetic link for IHPS. Overall, a heritability of 87% for IHPS was found [6].

Nitric oxide synthase gene (NOS1) could be a susceptibility locus for IHPS [7]. As nitric oxide (NO) is involved in smooth muscle relaxation, NOS must catalyze the production of NO, to allow the pylorus muscle to relax. It is suggested that reduced levels of NOS lead to a reduction in NO and hence diminish smooth muscle relaxation. As a result, increased muscular contraction occurs, leading to hypertrophy. In further support of this theory, Huang et al. [8] generated mice lacking NOS. These mice were found to have enlarged stomachs and hypertrophy of the pyloric sphincter. These findings could have great implications for future non-surgical treatment of IHPS, as early interventions to increase NO production could lead to management, if not prevention, of the disease.

Other factors implying genetic component are male preponderance as well as four times increased risk of a child of a mother who had IHPS to be diagnosed with the condition, compared to the child of a father who had IHPS.

Environmental Factors

In addition to genetic factors, a number of environmental factors have been implicated as risk factors for IHPS. Despite the higher incidence in males and the familial heritability pointing to a genetic cause, the decreasing prevalence in some countries such as Denmark points to environmental factors also playing a role in the development of IHPS [4]. Maternal smoking leads to infants having an increased likelihood of developing pyloric stenosis, probably due to interference with mitochondrial oxidative phosphorylation and increase in the concentration of calcium in pylorus muscle, via an unknown mechanism, leading to increased contraction [9]. Nonetheless, more research into the causal mechanisms is still needed. Furthermore, it could be argued that the decrease in incidence of pyloric stenosis in Denmark (from 1.4/1000 to 1.1/1000 live births) may be related to a possible decrease in consumption of cigarettes in Denmark due to increased health warnings in the media.

A further environmental factor that has received plenty of research and promising results is that of feeding methods for babies [10]. It has been implied that babies who are bottle-fed (with formula) are more likely to develop IHPS compared to those that are breast-fed.

Interestingly, Krogh et al. [11] conducted a study to compare feeding practices and identify their relationships, if any, with IHPS; 70,148 infants were involved in the study, and of that 65 had IHPS, 29 of which were bottle-fed for the first 4 months of life. When looking at the odds ratio, for those who were bottle-fed compared to breast-fed, it was 4.62, indicating that babies who were bottle-fed were 4.62 times more likely to suffer from IHPS compared to those who were breast-fed. Furthermore, when comparing babies who were both breast- and bottle-fed to those who were never breast-fed, latter group had a higher risk of developing IHPS. Additionally, McAteer et al. [12] found that, in a state in Washington, the incidence of pyloric stenosis had decreased from 14/10,000 to 9/10,000 while the likelihood of breast feeding had increased from 80% to 94%, therefore, supporting the findings of Krogh et al. [13]. The exact reasons for this are unknown; however, it has been argued that breast milk contains a number of peptide hormones that relax the pylorus, such as vasoactive intesti-

nal peptide (VIP), whereas formula milk has a higher gastrin content, which is thought to be involved in promoting smooth muscle contractions and could therefore be involved in pyloric muscle hypertrophy. However, these theories are still very tentative.

Other risk factors for pyloric stenosis are the first-born children (76.3% risk if first child, 19.6% if second), older maternal age, preterm delivery, caesarean section [13], and macrolide antibiotics among others. Interestingly, it was also found that if these risk factors were reduced, it also reduced the incidence of pyloric stenosis occurring in males and made the prevalence between males and females much more similar.

Metabolic Changes in Pyloric Stenosis

A fluid electrolyte imbalance is usually present in individuals with IHPS. Classically, child presents with hypochloremic, hyponatremic metabolic alkalosis. Hydrochloric acid is lost, resulting in metabolic alkalosis due to the loss of hydrogen and chloride ions via vomiting (chloride-responsive metabolic alkalosis). Usually, the parietal cells of the stomach secrete H^+ ions via carbonic acid, which cross the lumen membrane to enter the stomach. These are then transported to the duodenum via the spasmodic contraction of the pyloric muscle. However, in pyloric stenosis, this passage of ions from the stomach to the duodenum is not possible due to the anatomical changes, consequently preventing the duodenum from secreting pancreatic bicarbonate that is usually stimulated by the hydrogen ions. This lack of hydrogen ions further adds to the metabolic alkalosis [14]. Selective absorption of H^+ ions in the renal tubules due to metabolic alkalosis leads to loss of Na^+ and HCO3− further increasing hypokalemia and hyponatremia.

In children with prolonged fluid loss and vomiting due to delayed diagnosis, kidneys try to compensate for the fluid loss by reducing urine output. However, the kidneys also prevent the loss of potassium ions at the expense of hydrogen ions, due to a cation exchange. This causes a paradoxical aciduria. Hence, paradoxical aciduria is a sign of prolonged and severe dehydration.

Clinical Features and Differential Diagnosis

The main symptom of this condition, distinguishing it from other gastrointestinal conditions, is that of non-bilious, projectile vomiting after feeding which progressively worsens. Milk often changes from being white to becoming an off-white, pale yellow color, as it becomes curdled due to mixing with stomach acids whilst it remains stagnant in the stomach. The vomiting causes the metabolic changes of pyloric stenosis to manifest.

Additionally, infants with pyloric stenosis remain hungry after vomiting. Further symptoms relating to the inefficient digestion include a change in stools, with very small, "starvation stools" being produced. The reduced intake of food and nutrients inevitably leads to severe weight loss.

The longer the condition progresses, the more severe the symptoms become. If not treated in time, infant is severely dehydrated and has decreased urine output.

Differential diagnosis of pyloric stenosis should include vomiting due to over feeding and medical conditions like gastroesophageal reflux, sepsis, raised intracranial pressure, gastroenteritis, and other metabolic conditions. Surgical conditions mimicking symptoms of pyloric stenosis are rare and include pyloric web and pyloric atresia, which are usually diagnosed at the earlier age.

Investigations and Diagnosis

A diagnosis of pyloric stenosis is made clinically based on the symptoms and metabolic changes at presentation. A test feed is the common term used for the process of finalizing the diagnosis but currently it is becoming a vanishing clinical skill while sensitivity and specificity of ultrasound is approaching 100%. This involves examination of the abdomen while child is being fed, whereby a small "olive-sized" mass may be palpable in the epigastric region. This mass is the hypertrophic pyloric muscle, indicative of IHPS. Additionally, visible peristalsis in epigastrium might be present due to increased muscular contraction of the stomach and duodenum in an attempt to push food from the stomach downwards.

Imaging studies to confirm pyloric stenosis will most often include an ultrasound to assess the level of thickening of the pylorus. On most occasions, a muscle wall thickness of more than 3 mm (usually between 3 and 8 mm) on ultrasound investigation is diagnostic of pyloric stenosis with an average of 5 mm thickness in most cases [15]. Furthermore, a pyloric canal length of more than 14 mm and pyloric diameter of more than 12 mm are set as the parameters for diagnosing IHPS [16] (Figs. 4.1 and 4.2).

However, in an article published in the *Permanente Journal,* it was inferred that these parameters can be misleading as both the height and weight of infants can alter the pyloric thickness, although the thickness may be less than 3 mm for an infant of lower than average weight pyloric stenosis may still be present. This study clearly illustrates the importance of taking all factors into account when making a diagnosis, rather than relying solely on imaging techniques. A positive correlation can be seen between both age and weight of the infant with thickness of pyloric muscle [17].

Occasionally, a barium swallow is done, in which a characteristic "string sign" is found, illustrating the narrowing of the pylorus allowing very limited barium to travel from the

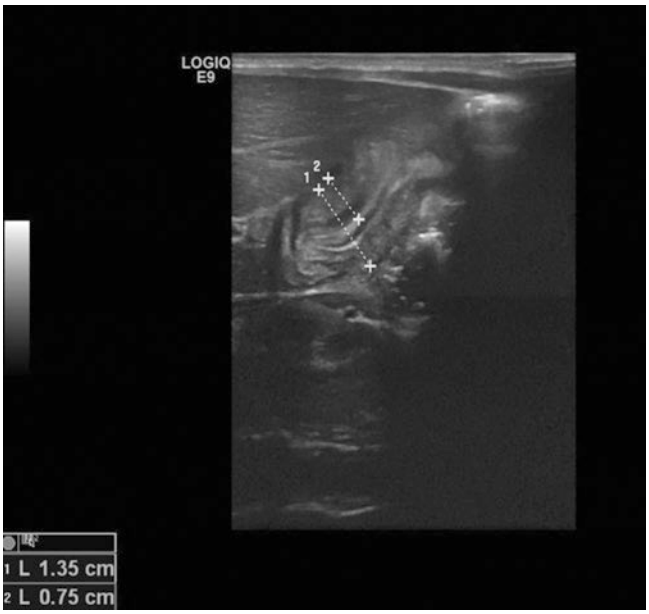

Fig. 4.1 Pyloric stenosis ultrasound transverse view

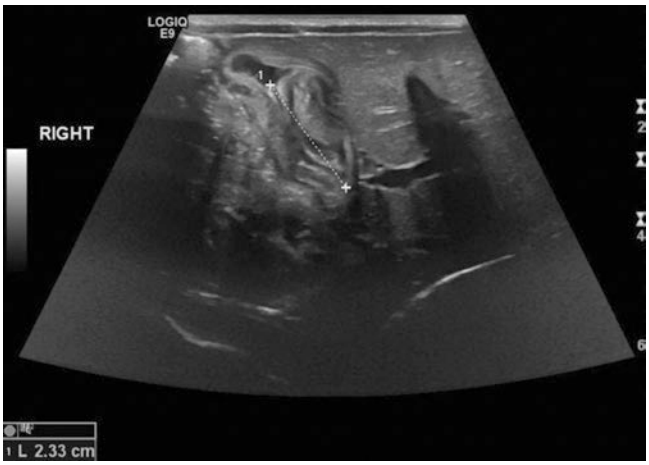

Fig. 4.2 Pyloric stenosis ultrasound longitudinal view

stomach to the duodenum. Other signs present on a barium swallow include the "shoulder sign" which occurs when the hypertrophied pyloric muscle indents into the pyloric antrum and the "mushroom sign" in which the hypertrophied pyloric muscle indents into the initial part of the duodenum.

Treatment

Preoperative Management

On suspicion of pyloric stenosis, the child should be kept nil by mouth. A nasogastric tube should be inserted and kept on free drainage. All fluid losses should be replaced using normal saline with 10 mmol/l of potassium.

Fluid resuscitation usually using 5% dextrose with 0.45% saline should be started. The amount should be adjusted after assessing the amount of dehydration. Once urine output is established, potassium should be added to IV fluid.

Surgical Treatment

Once the child is fully resuscitated, pyloromyotomy is the treatment of choice.

A pyloromyotomy is a simple procedure during which the hypertrophied pyloric muscle is cut longitudinally and then split. This releases the tension in the thick hypertrophied muscle and consequently allows the muscle to relax. This procedure is known as the Ramstedt's pyloromyotomy, as he initiated this technique of just spreading the muscle rather than doing pyloroplasty.

Surgery could be done either by right upper transverse abdominal incision or as the transumbilical pyloromyotomy (Bianchi approach) [18], which is performed via supraumbilical skinfold incision. Laparoscopic approach is also increasingly utilized. A number of studies have been conducted to establish the differences, if any, between the two. Nonetheless, most studies have suggested that both are effective, with different studies giving contradicting evidence on what form of procedure is more superior. In one study, it was suggested, however, that laparoscopic surgery is slightly superior in that babies are able to have a full feed in a slightly shorter duration of time compared to open pyloromyotomy (18.5 h versus 23.9 h), as well as having a shorter duration of stay in hospital (33.6 h versus 43.8 h) [19]. In a study by Lemoine et al. in 2011 [20], it has been suggested that a transumbilical pyloromyotomy is associated with increased postoperative pain compared to the laparoscopic approach.

In recent years the reports started emerging describing successful endoscopic treatment of IHSP, this novel procedure was adopted from gastroparesis treatment in adults. Gastric peroral endoscopic myotomy, known as G-POEM, totally defies the original principles of pyloromyotomy—the thickened pyloric muscle is accessed from the mucosa and at the end the mucosal entry is closed by clips. The advocates of this procedure suggest that patients may benefit from quicker recovery [21, 22] and it also can be applied treating older patients with atypical presentation [23].

Conservative Treatment

Medical treatment of IHSP with atropine sulfate was proposed a while ago and several meta-analyses were performed with a purpose to evaluate its efficacy [24–27]. The atrophine action on IHSP is based on its potent antimuscarinic activity and it leads to relaxation of smooth pyloric muscle.

Intravenous atropine is more effective than oral and produces a faster response but it also may be associated with adverse effects such as transient tachycardia and flushing. The latest comprehensive meta-analysis by Lauriti et al. detected five studies comparing atropine treatment with pyloromyotomy. Pyloromyotomy had higher success rate than atropine (100% versus 80.8%) and shorter hospital stay (5.6 days versus 10.3 days) [28]. Based on the findings, current recommendation in the UK is to reserve atropine treatment for patients unfit for general anesthesia or surgery.

Complications

Most common complications of surgery include inadvertent opening of the mucosa and incomplete myotomy. Other complications involve bleeding, wound infection, wound dehiscence, postoperative incisional hernia, and about 1% may undergo a negative laparotomy [29]. Self-limiting postoperative vomiting is considered a complication by some authors if hospital stay is extended beyond 48 h. Commonly postoperative vomiting is attributed to the degree of preoperative metabolic and electrolyte derangement and to a lower weight on admission [30]. In case of prolonged vomiting an incomplete pyloromyotomy should be suspected. As with many surgeries, wound infection is a further possible complication, more often seen at open surgery than at laparoscopy.

The in-hospital complication rate is reported between 2.7% and 5.16%. Complications of surgery are minimal, and in most cases, a pyloromyotomy is said to be very effective. A recent meta-analysis by Kelay et al. revealed a 4% higher rate of incomplete pyloromyotomy with the laparoscopic technique in comparison to open surgery. The incidence of mucosal perforation is calculated just about 1% and there was no significant difference between laparoscopic and open groups [31]. Nevertheless in the most recent papers laparoscopic surgery is being advocated as a superior method, it is supported by clinical evidence and excellent results reported in high volume centers [32, 33].

Outcome

Mortality in patients with pyloric stenosis may result from delays in diagnosis, followed by eventual dehydration and shock. The death rate for pyloromyotomy per se is extremely low, at about 0.1–0.5% but unnoticed intraoperative duodenal perforation may carry catastrophic consequences for a malnourished baby. The other cause of mortality can be perioperative apnea followed by cardiac arrest, which may occur as a sequence of profound or not sufficiently corrected metabolic alkalosis [31]. Therefore these patients should be placed on apnea monitor after surgery. IHPS is not considered as surgical emergency and metabolic correction should be performed before contemplating surgery.

The late outcomes of children who had pyloric stenosis remain unclear indicating that a long-term pediatric follow-up might be beneficial. Two studies suggested that neurological development of patients who had IHSP may be poorer in comparison to controls and the former IHSP patients might be more susceptible to functional abdominal problems later in life [34].

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Gastrointestinal Problems of the Newborn

5

Christophe Dupont, Nicolas Kalach, and Véronique Rousseau

Abbreviations

CF	Cystic fibrosis
DIC	Disseminated intravascular coagulation
EA	Esophageal atresia
GA	Gestational age
GER	Gastroesophageal reflux
GERD	Gastroesophageal reflux disease
GI	Gastrointestinal
MI	Meconium ileus
NEX	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
PPI	Proton pump inhibitors
SBS	Short bowel syndrome
SIDs	Sudden infant deaths
TEF	Tracheoesophageal fistula
VLBW	Very low birth weight

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GI Problems of Term Babies

Normal feeding is essential to growth and development of newborns: they are able to eat, to digest food, and to absorb nutrients, with normal bowel movements after being fed. Difficulties may occur, meaning a temporary adjustment or suggesting a more serious problem. Symptoms indicating gastrointestinal problems need to be recognized.

Difficult Feeding

The clinical evaluation of a neonate with complex issues related to feeding and swallowing includes a physical examination and feeding observation. Instrumental assessments of swallowing may be needed when concerns are noted regarding pharyngeal phase physiology and risks for aspiration with oral feeding. An interdisciplinary approach is often needed. Feeding difficulties may herald more severe and general disorders, listed in Table 5.1 [1].

Some diseases need urgent treatment. Hypothyroidism is diagnosed through a blood sample (low T3 and T4 and high TSH). Prader-Willi syndrome is characterized by hypotonia in an otherwise normal child and may require tube feeding at the beginning. Among a variety of craniofacial syndromes, Pierre Robin syndrome is characterized by retrognatism,

Table 5.1 Disorders that affect hunger/appetite, food-seeking behavior, and ingestion in newborns

Anatomic abnormalities of the oropharynx
Anatomic/congenital abnormalities of the larynx and trachea
Anatomic abnormalities of the esophagus
Disorders affecting suck-swallow-breathing coordination
Disorders affecting neuromuscular coordination of swallowing
Disorders affecting esophageal peristalsis
Mucosal infections and inflammatory disorders causing dysphagia
Other miscellaneous disorders associated with feeding and swallowing difficulties, such as xerostomia, hypothyroidism, trisomy 18 or 21, Prader-Willi syndrome, allergies, lipid and lipoprotein metabolism disorders, and a variety of craniofacial syndromes.

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glossoptosis, and sometimes cleft palate, with difficulties in breathing and swallowing which need immediate prevention measures, such as lying in prone position and tube feeding [1].

Gastroesophageal Reflux

Gastroesophageal reflux (GER) occurs when stomach contents backs up into the esophagus.

GER is most of the times benign, due to a high of volume inside the stomach, and/or temporary cardial insufficiency, and manifested only by regurgitations, spitting up and dribbling milk with burps or after feedings, without any other symptoms, especially pain. This situation characterizes the "happy spitters." These babies may be helped with feeding of smaller amounts and more frequent, burping the baby often during the feedings, holding the baby in an upright position for about 30 min after feeding and making sure that the baby's diaper is not too tight. When the child is not breastfed, using an infant formula enriched with food thickeners may also help [2].

GER may also be more severe. Several symptoms can be related to neonatal gastroesophageal reflux disease (GERD), Table 5.2. Feeding may be followed by forceful or projectile vomiting or spitting up of large amounts of milk. Reflux may also lead to esophageal irritation by the stomach contents, that is, esophagitis. When the stomach content reaches the pharyngeal regions, it can be aspirated into the lungs, a phenomenon recognized by "rattling," heard, and felt in the baby's chest and back. Babies may also gag and choke during feedings.

GERD may be primary, related to several mechanisms mainly of motor origin [2]. Variations during sleep and wakefulness suggest the involvement of autonomic nervous activity changes [3]. Signs and symptoms of GERD traditionally attributed to acidic reflux in neonates do not seem to be significantly altered by proton pump inhibitors (PPI) treatment in clinical trials [4] in contrast with their large use in clinical practice.

GERD may also be secondary, related to either medical of surgical disorders, Table 5.3. Food allergy dominates, especially for milk, to which the child may react either in infant formulas or in breast milk [5, 6], Diagnosis is based on elimination diets using milk free infant formulas or cow's milk elimination in mother's diet, since allergy testing is rarely positive at this age [5, 6]. The neonatal period remains a critical period for diagnosing conditions leading to vomiting, such as neonatal medical or surgical conditions. The latter must be kept in mind despite the wide use of prenatal diagnosis.

Table 5.2 Symptoms of neonatal gastroesophageal reflux disease (GERD)

Typical or atypical crying and/or irritability
Sleep disturbances
Apnea and/or bradycardia
Poor appetite; weight loss or poor growth (failure to thrive)
Apparent life-threatening event
Vomiting
Hematemesis and/or melena
Recurrent pneumonitis and/or pulmonary atelectasis.
Severe laryngomalacia

Diarrhea

In a newborn, the first bowel movements expel meconium, a sticky, greenish-black substance that forms in the intestines during fetal life. Yellow stools appear after the first few days. In breastfed babies, stools tend to be soft, seedy, yellow green, often as every few hours with feedings and at least several times a day. In formula-fed babies, stools are yellow and formed and occur once or twice a day.

In a baby with diarrhea, stools are watery, very loose, and occur very frequently. Signs of cramping are absent or difficult to perceive. Different causes may be considered, as indicated in Table 5.4 [7]. Diarrhea may reveal neonatal-onset Crohn's disease and intractable ulcerating enterocolitis [8] or neonatal enteropathies [9]. Several viral infections may be responsible, such as cytomegalovirus even in immunocompetent babies [10]. Other viruses may be involved [11], such as adenoviruses [12], parechoviruses [13], rotavirus, norovi-

Table 5.3 Secondary neonatal gastroesophageal reflux disease (GERD)

Medical disorders:
Milk allergy, in formula-fed infants or—in breast-fed children—to milk ingested by mothers
Other food allergies
Surgical conditions:
Esophageal atresia
Hiatal hernia
Diaphragmatic hernia
Omphalocele
Gastroschisis
Other rare causes of neonatal intestinal obstructions

Table 5.4 Frequency, etiology, and current management strategies for diarrhea in newborn infants [7]

Food allergy (20.5%)
Gastrointestinal infections (17.9%)
Antibiotic-associated diarrhea (12.8%)
Congenital defects of ion transport (5.1%)
Withdrawal syndrome (5.1%)
Hirschsprung's disease (2.5%), parenteral diarrhea (2.5%)
Cystic fibrosis (2.5%)
Metabolic disorders (2.5%)

Incidence 6.72 per 1000 hospitalized child, 39 cases of diarrhea (36 acute, 3 chronic); 3 patients died

rus, astrovirus, and some infections being potentially associated with necrotizing enterocolitis (NEC) [11].

Diarrhea in a newborn can quickly lead to severe dehydration and thus needs immediate oral rehydration. The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) solution contains 60 mOsm/L of Na and is recommended for children of Europe, but it seems to be effective in children living in developing countries [14]. Maintenance of breast feeding in breastfed infants is always recommended.

Constipation

Constipation is characterized by rare bowel movements, less than once a day, sometimes less than once every 3 or more days. Generally, stools are very compact and free from moisture, appearing as hard balls or pebbles. Signs of discomfort or pain are frequent, quick drawing-up of the legs, accompanied by a red-faced grunting as baby attempts to have a bowel movement. Examination of a constipated newborn needs checking the quality of feeding, the presence of abdominal distension, and gently analyzing the anal region.

Delay in passing meconium, abdominal distension, and low weight gain suggest or are associated with organic disturbances, such as Hirschprung's disease, often requiring a specific surgical procedure (see corresponding Chapters). Other severe presentation may need medical treatment such as meconium ileus (see below). The list of conditions potentially associated with constipation is depicted in Table 5.5 [15].

Functional constipation is the more likely. Breastfeeding newborn babies may pass three to four bowel movements per day within the first 2 weeks. The bowel movements of bottle-fed babies may be a bit less frequent. Apply a small dab of lubricating gel on baby's anus to protect the sensitive area and allow stool to pass a bit easier. Avoid using mineral oil as a lubricant.

Anal fissures are possible even in neonates, in the form a small tear in the anus. When limited, anal fissures may occur from baby forcing the passage of hard stool, usually with recurrent straining. Anal fissures may be associated with milk allergy [16]. What appears as a larger anal fissure may be an anomaly of the anal regions participating in the process of anorectal malformations, and requiring a surgical procedure.

Table 5.5 Key points of history and physical examination to guide in the evaluation of constipation in newborn [15]

Functional constipation
History
Starting during the first days of life
Obvious precipitating factors coinciding with the start of symptoms: fissure, change of diet, infections
Examination
Generally well, weight and height within normal limits
Soft abdomen
Normal appearance of anus and surrounding area
Organic constipation
Cystic fibrosis
Respiratory problems
Failure to thrive
Cow's milk allergy
Personal and family history (allergy)
Eczema
Hirschsprung disease
Onset of symptoms <1 month
Reported from birth or first few weeks of life
Passage of meconium >48 h
Bloody diarrhea, bilious vomiting
Growth delay or failure to thrive, bilious vomiting
Abdominal distension (sometimes massive)
Tight empty rectum in presence of palpable abdominal fecal mass
Explosive stool and air from rectum upon withdrawal of examining finger
Anatomic malformations
Anal stenosis: ribbons stools, tight anal canal on rectal examination
Abnormal anal position

Colic

Colic is a problem that affects many babies during the first 3 to 4 months of life, starting typically by 3 weeks of age but sometimes much earlier. It is defined as prolonged or excessive crying in an infant whose examination is normal, often associated with gas, irritability, and sleeping disorders. The crying can be very loud, can last for several hours a day, and predisposes to the shaken baby syndrome [17], hence the necessity for appropriate handling. What causes colic is still unclear. Studies show an increased fecal content of calprotectin [18], a marker of intestinal inflammation, thereby suggesting a physiological inflammation during the first months of life. Another possible reason for excessive crying in babies might be that they are oversensitive to gas in the intestine, similarly to what is observed in older children during irritable bowel syndrome, although this disorder is lacking evidenced-based approaches [19]. Milk allergy is also possible, thus leading to a trial elimination diet using cow's milk protein hydrolysates [6].

GI Problems of Preterm Infants

Feeding Difficulties

Digestive tolerance of preterm infants is one of the major problems of neonatal wards. Preterm infants have the paradoxical situation of a considerable demand in nutrients contrasting with a low digestive tolerance, owing to immaturity of the digestive tract, low oral sucking, and swallowing

maturity and with the need to interrupt as soon as possible parenteral feeding to reduce the infectious and metabolic risks and to introduce progressively enteral feeding to enhance the digestive tube maturity [20]. Although incompletely understood, difficulties in preterm infants in the neonatal intensive care unit (NICU) may be related to the potential consequences of an immature intestinal barrier defense and bacterial colonization disturbances, Table 5.6 [21]. What is at stake in this age range is the need to increase as far and as rapidly as possible the rate of enteral feeding with the constant need to avoid gastrointestinal signs of bad tolerance, especially trying to avoid NEC.

Table 5.6 NICU exposures and potential consequences on intestinal barrier defense and bacterial colonization in preterm infants [21]

	Exposure	Potential consequences
Non-specific barrier defense	*Prematurity*	Decreased immunoglobulin levels
		Decreased production of digestive enzymes
		Decreased production of mucus
		Dysfunctional peristalsis
	Delayed feeding	Villous atrophy
		Decreased production of digestive enzymes
		Decreased production of mucus
		Decreased peristalsis
	Medications	
	Histamine H2 blockers or proton pump inhibitors (PPI) Vasopressors and Indocin	Decreased gastric acidity
		Increased risk for intestinal ischemia and enterocyte injury
	Sedatives and paralytic agents	Decreased peristalsis
Bacterial colonization	*Prematurity*	Accentuated inflammatory response
		Abnormal glycosylation pattern
	Delayed feeding	Delay in bacterial colonization
	Broad-spectrum antibiotics	Prolonged sterilization of gut
		Delayed colonization of beneficial, commensal bacteria
		Preferred bacterial colonization of pathogenic bacteria
	Formula feeding and hospitalization	Preferred bacterial colonization of pathogenic bacteria

Progressive Increment of Oral Feeding in Premature Infants

Premature infants of gestational age (GA) >34 weeks are generally able to coordinate sucking, swallowing, and breathing, and so establish breast or bottle feeding. In less mature infants, oral feeding may not be safe or possible because of neurological immaturity or respiratory compromise [20]. In these infants, milk can be given as a continuous infusion or as an intermittent bolus through a fine feeding catheter passed via the nose or the mouth to the stomach [22]. In older babies, around 34 weeks of GA, the infant begins to suckle, and the bottle progressively replaces the tube feeding.

Several Cochrane reviews [23–26] confirm that the introduction of enteral feeding for very preterm infants, that is, less than 32 weeks of GA or very low birth weight (VLBW) <1500 g infants, is often delayed due to the bad clinical tolerance of early enteral introduction and may increase the risk of developing NEC. However, the available trial data suggest that introducing progressive enteral feeding before 4 days after birth and advancing the rate of feed quantities at more than 24 ml/kg/day does not increase the risk of NEC in very preterm infants and VLBW infants [23–26]. In contrast, prolonged enteral fasting may diminish the functional adaptation of the immature GI tract and extend the need for parenteral nutrition with its attendant infectious and metabolic risks [26]. Also, delayed introduction or slow advancement of enteral feeding results in several days of delay in the time taken to regain birth weight and establish full enteral feeds [26]. Trophic feeding, giving preterm infants small quantities of adapted preterm milk formulas to promote intestinal maturation, may enhance feeding tolerance and decrease the time taken to reach full enteral feeding independently of parenteral nutrition [23–26]. Although it is well agreed on that oral feeding should be initiated slowly first by the help of nasogastric tube and then progressively followed by oral feeding, the way in which the preterm infants are introduced and advanced varies widely. The use of dilute formula in preterm or VLBW infants might lead to an important reduction in the time taken for those infants to achieve an adequate daily energy intake [27]. Uncertainty also exists about the risk-benefit balance of different enteral feeding strategies in human milk-fed versus formula-fed very preterm or VLBW infants as the trials and reviews did not contain sufficient data for subgroup analyses [26].

Gastroesophageal Reflux (GER)

GER is common among preterm infants, due to several physiological mechanisms. Its real frequency in preterm and VLBW infants is not well established. The responsibility of

GER is suspected in the occurrence of apnea, bradycardia, pallor, cyanosis with or without oxygen desaturation, severe malaise, feeding difficulties with weight loss or poor growth (failure to thrive), crying, hematemesis, melena, and finally sudden infant deaths (SIDs) [2]. GER could be diagnosed for extra-digestive manifestations by 24 h pH-metry monitoring and for digestive manifestations by upper GI endoscopy with specific neonatal endoscopes [28].

The therapeutic management of GER still represents a controversial issue among neonatologists. Overtreatment, often useless and potentially harmful, is increasingly widespread. Hence, a stepwise approach, firstly promoting conservative strategies such as body positioning (the best position is the ventral decubitus associated with a 30° of orthostatism position under continuous monitoring in the NICU) or changes of feeding modalities should be considered the most advisable choice in preterm infants with GER [2]. Non-pharmacological management of GER might represent a useful tool for neonatologists to reduce the use of anti-reflux medications, that is, prokinetics and anti-H2 blockers or PPI, which should be limited, due to their side effects, to selected cases of severe symptomatic infants [29]. ESPGHAN Guidelines and summary of recommendations for GER diagnosis and treatment in infants are summarized in Table 5.7 [2].

Enteropathy

In neonatal units, there is a tendency to assume that any acutely sick infant with gastrointestinal symptoms has NEC, even though all digestive issues are not related to it. However, a better definition of neonatal enteropathy and of its risk factors in preterm neonates is needed. Neonatal enteropathy is the presence of feeding difficulties, with increased gastric residual, abdominal distension associated with sensitive and/or surgical abdominal examination, and rectal bleeding with bloody stool. Sometimes, preterm infants present with subocclusion (transient) or complete occlusion.

In a prospective study Suc et al. [30], 351 preterm infants admitted to a neonatal ward were fed similarly, depending their maturation, gestational age, and GI status. GI symptoms were seen in 53: 23 transient obstructions, 6 NEC, and 24 hemorrhagic colitis. Ten risk factors were found to be sig-

Table 5.7 ESPGHAN Guidelines; summary of recommendations for GER diagnosis and treatment in infants [2]

Recommendations	Quality of evidence
1. In infants, there is no symptom or group of symptoms that can reliably diagnose GERD or predict treatment response	B
2. Esophageal pH monitoring is a valid and reliable measure of esophageal acid exposure only	B
3. Combined multiple esophageal impedance-pH recording *is* superior to pH monitoring alone for evaluation of GER-related symptom association	B
4. Reflux-induced esophageal damage is defined endoscopically as visible breaks of the distal esophageal mucosa	C
5. There is no evidence to support an empiric trial of pharmacologic treatment in infants with symptoms suggestive of GERD	B
6. There is evidence to support a trial of an extensively hydrolyzed protein formula for a 2- to 4-week trial in formula fed infants with vomiting.	B
Thickening of formula results in decreased visible reflux (regurgitation)	A
7. Prone and lateral positioning is associated with a higher rate of sudden infant death syndrome. In infants from birth to 12 months of age, the risk of sudden infant death syndrome outweighs the potential benefits of prone sleeping. Therefore, supine positioning during sleep is generally recommended	A
8. In the infant with recurrent regurgitation, a thorough history and physical examination with attention to warning signs is generally sufficient To allow the clinician to establish a diagnosis of uncomplicated GER	C
9. In the infant with uncomplicated regurgitation, parental education, reassurance, and anticipatory guidance are recommended	C
10. In otherwise healthy infants with unexplained crying, notability *or* distressed behavior there is no evidence to support acid suppression	A
11. In the infant with reflux esophagitis, initial treatment consults of lifestyle changes and PPI therapy. In most cases, efficacy of therapy can be monitored by the degree of symptom relief.	A
12. In the infant with feeding refusal, acid suppression without earlier diagnostic evaluation is not recommended	D
13. In the vast majority of infants, reflux LS not related to pathologic apnea or to apparent life-threatening event, although a clear temporal relation exists in individual infants. In infant in whom this relation is suspected or if symptoms recur, impedance-pH recording in combination with polysomnographic recording may aid in establishing cause and effect	B

Level A: Consistent randomized controlled clinical trial, cohort study, all or none, clinical decision rule validated in different populations
Level B: Consistent retrospective cohort, exploratory cohort, ecological study, outcomes research, case-control study; or extrapolations from level A studies
Level C: Case-series study or extrapolations from level B studies
Level D: Expert opinion without explicit critical appraisal, or based on physiology, bench research or first principles

nificantly correlated with GI disturbances: umbilical venous catheter, benzodiazepines, birth weight <1,500 g, patent ductus arteriosus, ventilatory assistance, abnormal amniotic fluid, gestational age <32 weeks, early antibiotic treatment, passage of meconium >48 h, episodes of apnea, and/or bradycardia. GI problems might thus be separated into three groups: (1) isolated intestinal obstruction, seen in the most immature babies during the first week of life with the risk of developing NEC; (2) frank blood in the stool, indicating colitis and possibly minor forms of NEC; (3) combined obstructive and hemorrhagic symptoms, typical of NEC.

The diagnosis of enteropathy relies on clinical examination associated with biological parameters (complete blood count, C-reactive protein, and bacteriological cultures) and radiological exploration (abdominal X-ray and/or ultrasonography).

Specific treatment is offered according to diagnostic work up, that is, enteral and/or parenteral nutritional assistance, antibiotics, and sometimes support during acute life-threatening events.

Necrotizing Enterocolitis (NEC)

Background of Prematurity and NEC

NEC is a devastating gastrointestinal disease dominating in preterm infants. The pathogenesis, likely multifactorial, is incompletely understood. At this age, infants experience multiple perturbations to normal postnatal intestinal and immune development, all of which increase their vulnerability to NEC. The prevalence is increased in formula-fed infants, suggesting protection by the bioactive compounds of breast milk. The intestinal microbiota profiles observed during NEC as compared to control infants suggest a lack of benefits from commensal bacteria and an increased risk of intestinal inflammation and bacterial translocation by pathogenic bacteria [31]. NEC incidence seems also reduced by prebiotics and probiotics and increased after prolonged antibiotics exposure use leading to delayed bacterial colonization, with preference for pathogenic microorganisms. H2-blocker or PPI, decreasing gastric acidity, might dampen one component of the first-line defense against pathogenic antigens provided by intestinal tract [32].

The role of Toll-like receptor-4 (TLR4) is likely to be critical in the postnatal susceptibility to NEC of preterm infants [33]. In animal models, the absence of TLR4, such as in TLR4 knockout mouse, prevented the development of NEC [34]. Downregulation of TLR4 suppresses downstream pro-inflammatory signaling, such as observed with postnatal intestinal bacterial colonization by commensal organisms, whereas TLR4 activation increases pro-inflammatory signaling.

Signs and Symptoms

NEC affects typically premature infants, with a timing of onset generally inversely proportional to the GA of the child. Initial symptoms include feeding intolerance, increased gastric residuals, abdominal distension, and bloody stools. Symptoms may progress rapidly to abdominal discoloration with intestinal perforation and peritonitis and systemic hypotension requiring intensive medical support [35].

Spontaneous ileal perforation (SIP) is a clinical entity distinct from NEC, consisting of a perforation in the gastrointestinal tract of a newborn with no demonstrable cause, typically found in the terminal ileum, with abdominal distention and vomiting for 3 to 4 days as presenting symptoms. SIP is frequently seen in preterm newborns, especially with very low birth weight, and rarely in full-term newborns. The potential protective effects for SIP are the use of caffeine, inhaled nitric oxide, and early transfusion [36].

Diagnosis

The diagnosis is usually suspected clinically but often requires the aid of diagnostic imaging modalities. Radiographic signs of NEC include dilated bowel loops, paucity of gas, a "fixed loop" (unaltered gas-filled loop of bowel), pneumatosis intestinalis, portal venous gas, and pneumoperitoneum (extraluminal or "free air" outside the bowel within the abdomen). More recently ultrasonography has proven to be useful as it may detect signs and complications of NEC before they are evident on radiographs [37]. Recently, fecal biomarkers, such as fecal calprotectin, have been tested as noninvasive markers, for diagnosis and follow-up but current results show fecal calprotectin levels in at-risk infants highly variable [38, 39].

Three stages of NEC exist:

Stage 1: Apnea, bradycardia, lethargy, abdominal distension, and vomiting
Stage 2: Pneumatosis intestinalis and the above features
Stage 3: Low blood pressure, bradycardia, acidosis, disseminated intravascular coagulation (DIC), and anuria

Treatment

Primary treatment consists of supportive care. Bowel rest is obtained by stopping enteral feeds, intermittent suction to obtain gastric decompression, fluid repletion to correct electrolyte abnormalities and third space losses, adapted support for blood pressure, parenteral nutrition, and prompt antibiotic therapy. Monitoring is clinical, although serial supine and left lateral decubitus abdominal roentgenograms should be performed every 6 h. Where the disease is not halted through medical treatment alone, or when the bowel perforates, immediate emergency surgery to remove the dead

bowel is generally required. Surgery may require a colostomy, which may be able to be reversed at a later time. Some children may suffer later as a result of short bowel syndrome (SBS) if extensive portions of the bowel had to be removed.

Prevention

The American Academy of Pediatrics, in a 2012 policy statement, recommended feeding preterm infants human milk, finding "significant short- and long-term beneficial effects," including lower rates of NEC. Meta-analyses of four randomized clinical trials performed over the period 1983 to 2005 supports the conclusion that feeding preterms human milk is associated with a significant reduction (58%) in the incidence of NEC. A more recent study of preterm infants fed an exclusive human milk diet compared with those fed human milk supplemented with cow-milk-based infant formula products noted a 77% reduction in NEC [40].

Parenteral nutrition (PN) provides a relatively safe means of meeting nutrient intakes, and while sparing the intestine and is widely used in preterm infants in the initial period after birth, especially in infants who do not tolerate enteral feeds. In most preterm infants, authorities recommend amino acid intakes approximating to 3.5–4 g/kg/day of protein, lipid intakes of 3–4 g/kg/day, and sufficient carbohydrate to meet a total energy intake of 90–110 kcal/kg/day [41]. However, in the prevention of NEC, the short-term benefits of parenteral nutrition itself or of variation in its composition lack evidence.

Neonatologists from NICU reported on the importance of providing small amounts of trophic oral feeds of human milk starting, while the infant is being primarily fed intravenously, in order to prime the immature gut to mature and become ready to receive greater oral intake [40]. Human milk from a milk bank or donor can be used if mother's milk is unavailable [40]. Finally, probiotic and prebiotic supplementation is a promising approach for the prevention of NEC in preterm and VLBW infants [42].

Typical recovery from NEC if medical, nonsurgical treatment succeeds includes 10–14 days or more without oral intake and then demonstrated ability to resume feedings and gain weight. Recovery from NEC alone may be compromised by comorbid conditions that frequently accompany prematurity. Long-term complications of medical NEC include bowel obstruction, anemia, and SBS.

Meconium Ileus (MI)

Definition and Etiology

Meconium ileus (MI) results from an intraluminal intestinal obstruction produced by thick inspissated meconium. Most patients have cystic fibrosis (CF) disease (90% of cases), others having a history of isolated simple MI. The abnormal meconium is very dry, contains higher-than-usual concentrations of protein, and adheres firmly to the mucosal surface of the distal small bowel, creating an intraluminal obstruction [43]. This leads to poor intestinal motility, low grade obstruction, and distended loops without air fluid levels. Associated risk factors are severe prematurity and low birth weight, caesarean delivery, maternal $MgSO_4$ therapy, and maternal diabetes. The incidence of MI has shown to increase while its management continues to be challenging and controversial for the risk of complicated obstruction and perforation [44].

Diagnosis

MI is usually manifested by intestinal obstruction. This situation is more common in the very preterm and VLBW infants than preterm infants more than 32 weeks GA. Clinically, in an otherwise healthy-appearing infant, abdominal distension is visible in the first 12–24 h of life, without meconium elimination in the first 48 h. Physical examination reveals firm palpable masses throughout the abdomen without real surgical signs. There may be feeding difficulties with increased gastric residuals but without vomiting.

MI may be complicated in utero by volvulus, atresia, perforation, and meconium peritonitis, in 30% to 50% of the cases. When born with those complications, infants appear sicker, with often vomiting and signs of neonatal sepsis and more marked abdominal distension causing respiratory distress.

Radiological examination shows dilated bowel loops and the viscous nature of meconium produces a "ground-glass" appearance. Perforation after birth results in free intraperitoneal air. Newborns suspected of MI or any other distal bowel obstruction are diagnosed with a contrast enema study.

All newborns with MI need to be assessed for CF with the sweat chloride test and the genetic assessment of the several mutations of CFTR CF [43].

Treatment

In case of simple MI, approximately 60% of infants have their obstruction successfully relieved by diagnostic contrast enema, ideally using a water-soluble contrast agent [43]. Failure of the contrast to dislodge the inspissated meconium after two attempts is an indication for surgical intervention, for enterotomy with acetylcysteine irrigation and immediate closure. When the enema fails, acetylcysteine, 5 ml every 6 h, may be given via a nasogastric tube to help complete the clean-out. Complicated MI always requires surgical interventions, and the choice of operations depends on the pathologic findings [43].

Congenital Anomalies

Esophageal Atresia (EA)

Definition

Esophageal atresia (EA) (also dealt with in Chap. 3) is a congenital anomaly of the tracheoesophageal separation where the development of the midst esophagus is lacking and that occurs either rarely isolated or frequently associated with trachea-esophageal fistula (TEF). In the most frequent case (85%) the trachea-esophageal fistula is located in the distal esophagus, Fig. 5.1, Table 5.8 [45–47]. The incidence of EA varies from 1 in 3000 to 1 in 4000 live births, with a slight predominance in male and in preterm infants. The role of genetic remains unclear.

Diagnosis

In approximately one-third of pregnancies with EA, the presence of polyhydramnios, due to the inability of the fetus to swallow amniotic fluids, may suggest the disease. Antenatal ultrasound then may show, after about 26 weeks of gestation, an absent or small stomach, which in the setting of polyhydramnios strongly suggests EA. The upper neck pouch sign is another sign that may help antenatal diagnosis.

In almost all types of EA, a feeding tube will not pass through the esophagus at the first neonatal exam in the delivery room. Within first hours the newborn will present oral secretion followed by severe cough and choking episodes. The infant may become cyanotic and may even stop breathing as the overflow of fluid from the blind pouch is aspirated the trachea. If cyanosis results in laryngospasm (basically a protective mechanism to prevent aspiration into the trachea), a severe respiratory distress will rapidly develop.

Digestive symptoms occur immediately. Infants vomit when they are fed, and, owing to the passage of air through the distal trachea-esophageal fistula, abdominal distension develops. A flat or a gasless abdomen suggests an EA without fistula. The most dramatic presentation of the trachea-esophageal fistula occurs with a proximal fistula. Affected infants develop life-threatening respiratory failure from aspiration almost immediately after birth. In contrast, with the so-called H-type, infants usually do not develop any symptom in the neonatal period, but present later on with a history of recurrent mild respiratory distress related to feeding or pneumonia.

Other birth defects may coexist in other organs, such as in the VACTERL association involving other organs (Vertebral column, Anorectal, Cardiac, Tracheal, Esophageal, Renal, and Limbs).

If any of the above signs/symptoms or malformation is noticed, it is mandatory to pass a catheter into the esophagus to check, prompting other tests to confirm the diagnosis if a resistance is noted. This can be done with a catheter visible on a regular X-ray film, demonstrating the blind pouch ending. A small amount of barium placed through the mouth may help.

Treatment

If EA or trachea-esophageal fistula is suspected, all oral feedings are stopped and replaced by intravenous fluids. Appropriate positioning will help the infant draining the secretions and decrease the risk of aspiration. A tube with a continuous aspiration placed into the proximal esophagus

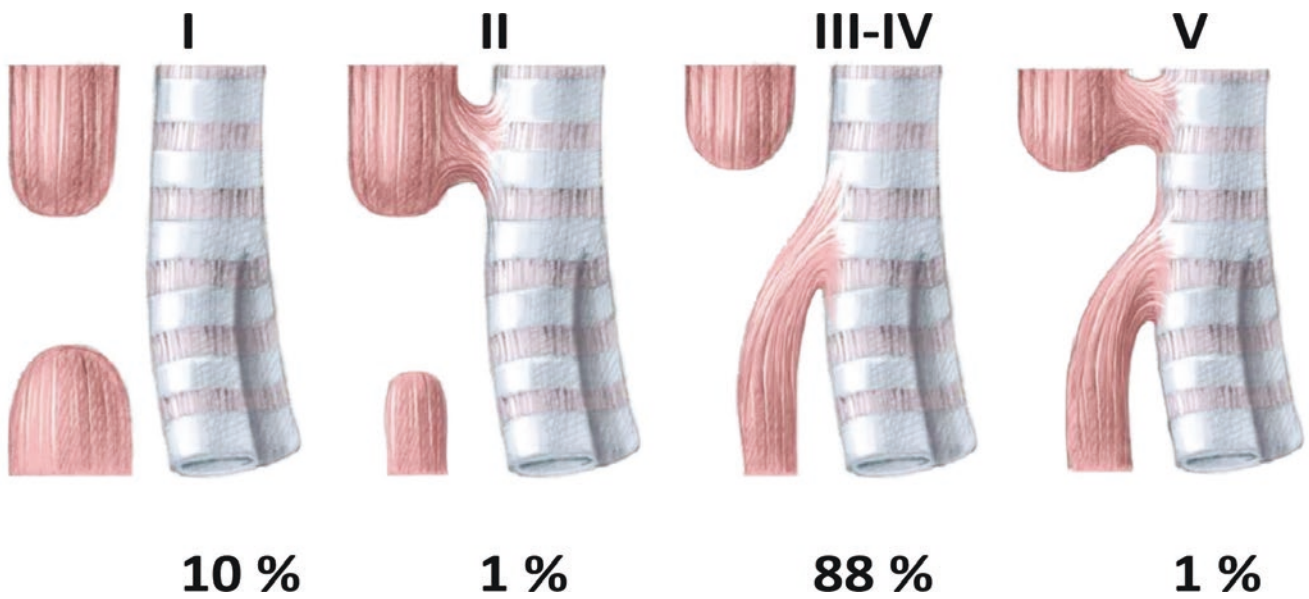


Fig. 5.1 The different type of esophageal atresia. (Dessins: Reinhold Hankel, in *Pediatric Surgery* © Springer-Verlag 2006)

Table 5.8 Classification of esophageal atresia [45–47]

Gross et al. [45]	Vogt et al. [46]	Ladd et al. [47]	Name	Description	Frequency
	Type 1		Esophageal agenesis	Very rare complete absence of the esophagus, not included in classification by Gross or Ladd	N/A
Type A	Type 2	I	"Long Gap," "Pure," or "Isolated" esophageal atresia	Characterized by the presence of a "gap" between the two esophageal blind pouches with no fistula present.	7%
Type B	Type 3A	II	Esophageal atresia with proximal TEF (tracheoesophageal fistula)	The upper esophageal pouch connects abnormally to the trachea. The lower esophageal pouch ends blindly.	1%
Type C	Type 3B	III, IV	Esophageal atresia with distal TEF (tracheoesophageal fistula)	The lower esophageal pouch connects abnormally to the trachea. The upper esophageal pouch ends blindly.	86%
Type D	Type 3C	V	Esophageal atresia with both proximal and distal TEFs (two tracheoesophageal fistulas) TEF (tracheoesophageal fistula) only with no esophageal atresia	The upper and lower esophageal pouches make and abnormal connection with the trachea in two separate, isolated places.	2%
Type E	Type 4		H-type	Esophagus fully intact and capable of its normal functions, however	4%
Type F			Congenital esophageal stenosis	A congenital form of esophageal stricture Esophagus is fully intact and connected to the stomach, however, the esophagus gradually narrows, causing food and saliva to become "caught" in the esophagus On occasion, this type can go undiagnosed until adulthood Not included in classification by Vogt or Ladd	N/A

N/A Not known

pouch can minimize the aspiration of saliva. Also, minimizing positive pressure ventilation can minimize gastric distension and reflux. The use of systemic anti-H2 or PPI can reduce the toxicity of acid secretion on the lung and the distal esophagus pouch, but reflux in itself might help the development of the lower pouch. If gastric distension occurs progressively, surgery becomes an emergency.

Surgery to fix EA is a semi-emergency. Once the baby is in condition, surgery is made by thoracotomy, thoracoscopy, and robot surgery. The esophagus can usually be sewn together. Following surgery, the baby may be hospitalized for a variable length of time depending mainly on associated malformations. Care for each infant is individualized [47]. Long-term follow-up is mandatory, owing to the risk of chronic reflux, Table 5.3.

Imperforate Anus

Definition

Anorectal anomalies occur in 1 out of 2500–5000 births, slightly more commonly in males and with associated anomalies in more than half of affected infants. These are vertebral, genitourinary tract, and GI malformations. Imperforate anus may occur in the setting of the VACTERL association (see Chap. 11, EA). The anatomical variability of these anomalies renders genetic analysis complex, so that genetic factors are clearly associated with anorectal anomalies in only 8% of patients. In infants with trisomy 21% and 18%, 95% of those with anorectal malformations have imperforate anus without fistula, as opposed to only 5% of all patients with anorectal anomalies.

Imperforate anus is the most common anomaly, but other forms of anorectal malformations may occur, such as anterior ectopic anus [48]. This form is more commonly seen in females and presents with constipation [49].

Diagnosis

Anorectal malformations have been classified according to the level of the rectal pouch, that is, "high," above the elevator sling, "low," below the elevator sling, or "intermediate" and to the presence or the absence of an associated fistula. The latter is present in 95% of cases, either externally as an ano-cutaneous fistula or internally as a recto-urinary tract fistula [50].

Diagnosis relies on clinical examination of the perineal region at birth, completed with X-ray studies in cases of diagnostic uncertainty and for the assessment of the associated malformations.

Females may pass meconium through a perineal, vestibular, or vaginal fistula. Infants with a "low" defect may have cutaneous fistulas, meconium present at the perineum, a bulging anal membrane, and well-formed buttocks. Males with a "low" defect may have a bucket-handle malformation or cutaneous fistulas along the midline raphe toward the penis, discharging meconium drops. Finding meconium in the urine or air in the bladder may identify the presence of an internal fistula [50].

Plain abdominal X-ray may show progressive distal bowel obstruction. To identify the distance between the rectal pouch and the skin, a cross-table prone film is obtained with the pelvis elevated, after the first 24 h of life, during which the gap may appear falsely increased. Perineal ultrasound examination may prove to be more accurate than plain films. Abdominal ultrasound, cardiac echography, and skeletal films may be required to rule out other associated anomalies.

Treatment

Several procedures aim at restoring the anorectal canal in "low" defects, by cut-back of a cutaneous fistula, anal transposition, or limited posterior sagittal ano-rectoplasty.

"High" and so-called intermediate defects require an immediate colostomy, followed by elective repair of the localized fistula. Several approaches to the definitive correction of the anorectal anomalies have been described; the aim is always to close all fistulas and then tunnel the rectal pouch through the anatomic sphincter muscle to the anoderm. A posterior sagittal ano-rectoplasty is often the choice.

Children born with "low" defects often will be constipated, whereas incontinence may occur in one-third of patients with "high" defects. Quality of life correlates closely with whether continence can be established. Nevertheless, most of those infants can be rendered functionally continent through a bowel management program with medical laxative treatment, anal enema, and anorectal biofeedback rehabilitation program.

Abdominal Wall Problems

Umbilical Hernia

Umbilical hernia is in infants and is manifested by the intestine protruding through an opening in the abdominal muscles in the navel region. In an infant, it may be hernia may be especially evident when the infant cries, causing the baby's bellybutton to protrude. Many umbilical hernias close on their own by age 1, though some take longer to heal. Surgical repair to prevent complications is needed only when umbilical hernias do not disappear by age 3.

Omphalocele

An omphalocele is an abdominal wall defect where the intestines, liver, and other organs remain in a sac outside of the abdomen because of a defect in the development of the muscles of the abdominal wall. Omphalocele occurs in 2.5/10,000 births and has a high rate of mortality (25%) and of association with other severe malformations, cardiac anomalies (50%), and neural tube defect (40%) and may be associated with pulmonary hypoplasia. The digestive omphalocele can be associated with Beckwith-Wiedemann syndrome. Chromosomal abnormalities are seen in approximately 15% of live-born affected infants, such as trisomy 13 or 18. Long-term complications include parenteral nutrition dependence, GER, parenteral nutrition–related liver disease, feeding intolerance, and neurodevelopmental delay, owing to the difficulties encountered during intensive care and long-lasting stay in the hospital [51]. Omphlocele is usually detected during routine ultrasonographic surveillance, during an investigation of a disparity of uterine size with time from conception or during an evaluation of an increased maternal serum alpha-fetoprotein level. Death rate amounts to 15% of cases and in giant malformations including liver.

Gastroschisis

Gastroschisis is a congenital defect of the anterior abdominal wall, usually less than 4 cm, almost always to the right of the umbilicus, through which the abdominal contents freely, protrude. There is no overlying sac as opposed to omphalocele (which involves the umbilical cord itself and where the organs remain enclosed in visceral peritoneum). The defect may be due to a disruption of the blood supply of the abdominal wall in the first weeks after conception. Antenatal ultrasound examination has made the detection of gastroschisis possible in the first trimester of pregnancy. Surgical management at the birth is an emergency and depends on the presence of perivisceritis. It may require numerous surgical procedures. The neonatal management needs to be careful and done by trained specialists early minimal enteral feeding and gradual enteral nutrition increment and progressive refeeding considerably improve the prognosis [52].

Prune-Belly Syndrome

Prune-belly syndrome is a group of birth defects that involve three main problems, a poor development of the abdominal muscles, causing the skin of the belly area to wrinkle like a prune, cryptorchidism, and urinary tract problems. The latter are the most common issue in the long-term outcome of affected patients.

Inguinal Hernia

Inguinal hernia occurs when the contents of abdominal cavity protrudes through the inguinal canal. Inguinal hernia is very frequent, affecting predominantly male infants, especially premature infants. Inguinal hernia repair in infants is a routine surgical procedure. However, numerous issues, including timing of the repair, the need to explore the contralateral groin, use of laparoscopy, and anesthetic approach, remain unsettled [53].

Omphalomesentéric Band

The omphalomesenteric cord (band) represents the distal residual of the omphalomesenteric duct and may be an origin of obstruction in early infancy with various radiographic pre-

sentations, including intermittent obstruction [54]. The treatment requires surgery.

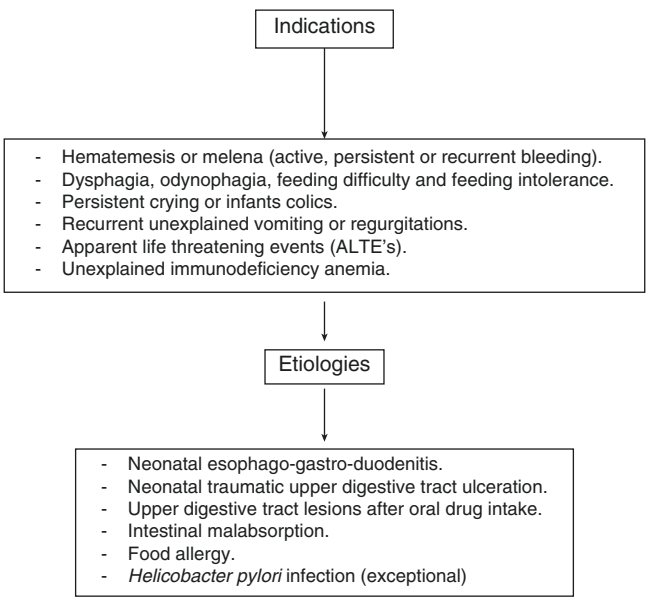
Upper and Lower Endoscopy

Upper Digestive Neonatal Endoscopy

Endoscopy is usually performed at the practitioner's request, upon clinical symptoms that may be considered symptomatic of endoscopic lesions, affecting the sole esophagus or also the stomach, Table 5.9. These are mainly hematemesis, of course, but also retching, regurgitation, increased crying, anorexia, difficult feeding and/or failure to thrive, and malaise. In a multicentric study [55], a large range of symptoms led to endoscopy, hematemesis (55.5%), malaise-like sudden pallor, bradycardia, or cyanosis with or without hypotonia (39.4%), feeding problems and vomiting (53.3%).

Upper bleeding is considered to usually affect sick premature infants and to be unusual in term healthy newborns. In 100 infants treated in an NICU [56], 20% showed gastrointestinal bleeding and mechanical ventilation was the only risk factor. Several studies described healthy full-term neonates who, after an uneventful delivery, had more or less profuse bleeding in the first 48 h of life. In a case-control study comprising 5180 newborn babies, 64 (1.23%) suffered from upper gastrointestinal bleeding [57]. In the case-control study recently published in neonates with upper gastrointestinal bleeding [57], esophageal damage was observed in 24/53 patients. Esophageal lesions were isolated in nine cases and occurred jointly with gastric or duodenal damage respectively in 14 and 1 cases. Gastric lesions were seen in 43/52 patients and duodenal ones in 1/52. There were 17 gastric ulcers and 1 duodenal ulcer [57]. Massive bleeding with life-threatening proportions in the first 24 h of life may allow discovering hemorrhagic gastritis, profuse upper gastrointestinal bleeding, and duodenal ulcers. Antenatal bleeding seems also possible, with esophagitis and gastritis revealed by a bloody amniotic fluid [58]. Fatalities during the first days of life resulting from bleeding due to gastric ulceration have also been described [59]. However, the exact incidence of endoscopic lesions in newborns remains debatable. Endoscopic examination of newborns is rarely readily available, and even severe lesions in the upper GI tract probably often remain undiagnosed or are not detected until the condition of the patient deteriorates because of perforation or hemodynamically significant bleeding. Also, owing to the abovementioned lesions, already well known by neonatologist, the use of PPI or anti-H2 drugs in neonates at risk may have considerably reduced the actual occurrence of these lesions.

Table 5.9 Indications and etiologies of upper digestive endoscopy in the neonate



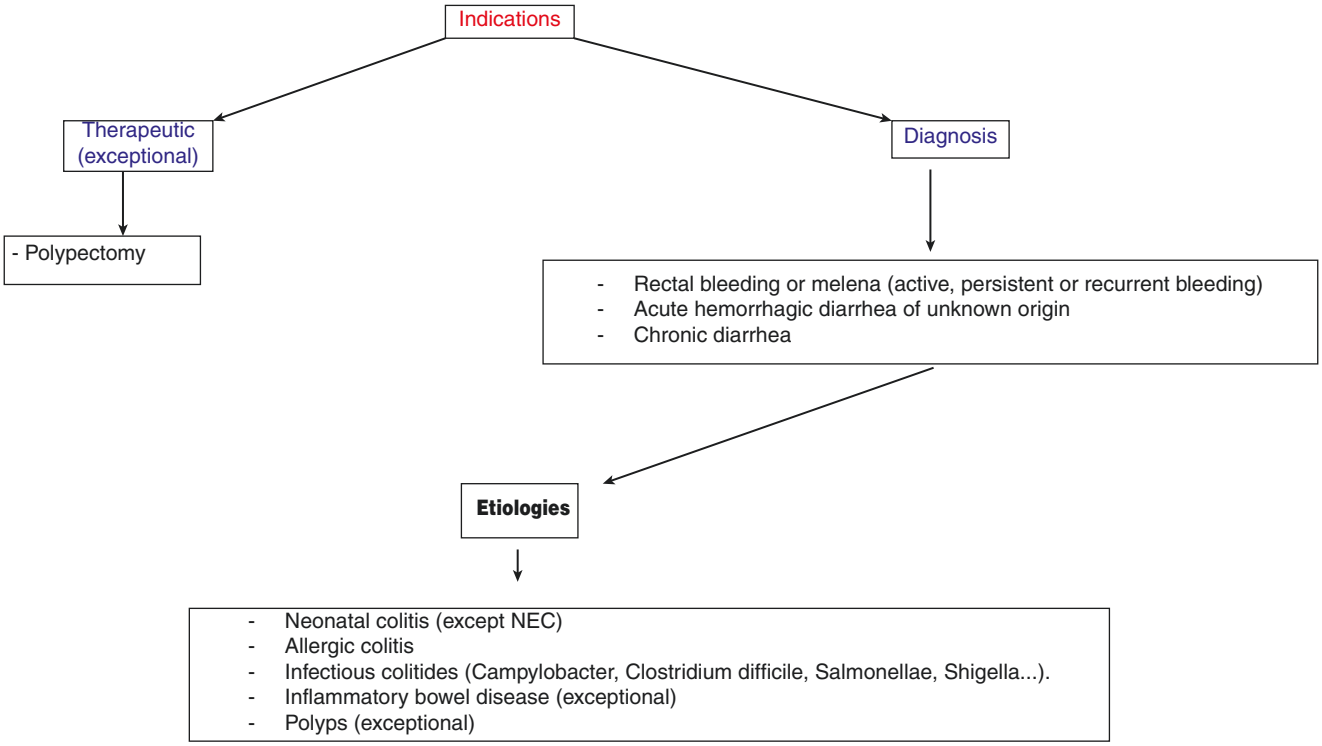
Endoscopy may also be prompted by severe general symptoms, in the form of apparent life-threatening events (ALTEs) led to discovery of severe lesions of esophagus and stomach in two different neonatal cases [60, 61]. If severe esophago-gastritis lesions are shown, an antacid treatment can control the symptoms and be perhaps lifesaving. In addition, the disease has a spontaneously favorable outcome ruling out the need for home monitoring and alleviating considerably the strain on the family. In the presence of neonatal ALTEs, upper digestive endoscopy might be proposed as part of the initial work-up, along with other mandatory investigations, such as diagnosis of long QT syndrome. This does not preclude the use of other means of investigation of GER, such as pH-metric esophageal recording.

Mucosal lesions seen at endoscopy may affect the esophagus, in the form of severe esophagitis appearing as ulcers covered with fibrinous material, occupying the whole perimeter of the esophagus and up to one-third or two-thirds of its distal part. The cardial orifice is usually constantly open with a frequent local inflammation. Gastric lesions usually appear in the form of petechiae or aphteous ulcers, associated or not to blood in the lumen. Duodenal lesions are limited to erythema, mucosal ulcers being rare. In the group with esophagitis and gastritis, severe mucosal lesions could be found following minor symptoms.

Lower Digestive Neonatal Endoscopy Findings (Colonoscopy)

In infants, rectal bleeding may be confusing when it is due to swallowed maternal blood during delivery or during breastfeeding, Table 5.10. The stool may be black or bright red and

Table 5.10 Indications and etiologies of lower digestive endoscopy in the neonate



the infant without symptoms. The Apt-Downey test distinguishes fetal from maternal hemoglobin and helps distinguishing the source of bleeding. Hemorrhagic disease of the neonate is now uncommon after prophylactic administration of vitamin K at delivery. Finally, maternal drug such aspirin, phenytoin, cephalotin, and phenobarbital may interfere with clothing function and cause hemorrhage [62]. Anal fissures are regarded as the most common cause. Giacoia and Williams [63] report two cases in which the presence of an anal fissure delayed the diagnosis of nonspecific colitis in premature infants. It is the authors' experience that the aspect of anal fissure in the term or preterm neonate is often the exteriorization of a rectal inflamed mucosa and that such a diagnosis should probably prompt the systematic search for an underlying condition, such as colitis [28], and, as mentioned above, cow's milk allergy [16].

Rectal bleeding in the neonatal period is nonetheless an alarming event that suggests possible NEC, the latter being associated with a string of other clinical features which may render the diagnosis likely and the need for urgent hospital care and investigation. Early reports, which lack reliability in the absence of endoscopy, had already addressed the issue of rectal bleeding and NEC. In a 1979 survey of 69 infants referred to hospital for rectal bleeding in the first month of life, in which NEC accounted for only 3% of cases [64]. Six infants born at term and had normal perinatal courses developed an inflammatory proctocolitis in the first month of life while being exclusively breast fed and had sigmoidoscopy showing focal ulcerations, edema, and increased friability [65]. A personal study [66] aimed at a better appraisal of unexplained neonatal rectal bleeding using rectosigmoidoscopy. Mucosal alterations were ecchymotic patches, either in the form of one or several longitudinally stretched ecchymotic stripes on an otherwise normal mucosa or in the form of shapeless ecchymosis irregularly dispersed on a friable and congestive mucosa. In 18 neonates with rectal bleeding, colonoscopy revealed a colitis characterized endoscopically by ecchymotic mucosal lesions, the so-called ecchymotic colitis [67].

Colonoscopic changes during the neonatal period may involve NEC, *Campylobacter* infection, *Clostridium difficile*, and dietary protein intolerance [28]. Rectal bleeding and/or colonoscopic changes have also largely been associated with blood transfusion or exchange transfusion [28]. Ulcerative colitis, a rare condition during infancy [28], may be recognized on the association to rectal bleeding and colonoscopic lesions of a severe and progressively worsening alteration of the general condition, Table 5.10.

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Enteral Nutrition in Preterm Neonates

6

Gianluca Terrin, Maria Di Chiara, Giulia Sabatini, Thibault Senterre, and Mario De Curtis

Introduction

Over one million children die from preterm birth-related complications every year. According to the World Health Organization, there has been an increase in the incidence of preterm births over the past 20 years [1]. Preterm births are the leading cause of newborn deaths and the second leading cause of death in children under the age of 5 years. Preterms have higher rates of adverse health outcomes in early adulthood compared with their term counterparts [1, 2]. Malnutrition of preterm newborns, especially those very low birth weight (VLBW), is associated with poorer head growth, persistent smaller head size resulting in poor psychomotor and mental skills, higher rates of cerebral palsy, and autism [3]. Despite significant improvement in neonatal intensive care, nutrition of preterm infants remains a challenging issue. Enteral nutrition (EN) should be attempted in each preterm neonate as soon as possible [4, 5]. However, the gastrointestinal tract of preterm infants and especially of VLBW infants is immature at birth and initially incapable of receiving full enteral feeding [5–7]. The inability of receiving EN is defined feeding intolerance (FI), which is variously characterized by signs such as increased volume gastric residual, bilious- or blood-stained stomach residuals, abdominal distension, and/or emesis [8–10].

In most cases, FI represents a benign condition related to the immaturity of gut function; however, its presentation largely overlaps with that of an impending necrotizing enterocolitis (NEC) [11–16]. NEC is a major gastrointestinal emergency in preterm newborns and affects about 3–10% of VLBW infants [16]. Early enteral feeding practices are potentially modifiable risk factors for NEC in very preterm infants. In order to limit the occurrence of NEC, EN is often delayed or withheld over long periods, leading to a delay establishment of full enteral feeding. This practice may prolong the time to regain birth weight and duration of hospital stay. Human milk (HM) and preterm formula represent the two available options for enteral nutrition in preterm newborns. Even if HM is the most physiologic choice, it does not address nutritional requirement of preterm infants. On the other hand, the use of formula is associated with an increased risk of NEC. For all these reasons, optimal use of EN in preterm newborns is still a challenging issue.

In this chapter, we focused on the peculiarities of the nutritional care of preterm neonates, during hospitalization and after discharge from neonatal intensive care unit (NICU).

Nutrition Objectives in Premature Neonates

The vast majority of infants born at a gestational age lower than 29 weeks do not achieve the median birth weight of the reference fetus at theoretical term or 40 weeks postmenstrual age (PMA) [17]. At discharge, the estimates of growth failure range from 30% to 67%. EN should be introduced appropriately to reduce the risk of cumulative nutritional deficit and malnutrition. However, it remains unclear which postnatal artificial feeding regimens are appropriate to support postnatal growth adequately [5–8].

Age of life and clinical conditions influence modalities of enteral nutrition administration. Two different phases could be identified: (I) the early adaptive period of clinical instability (from birth to approximately day 7 of life) and (II) the intermediate-stable growing period. Recommended intakes and modalities of enteral feeding during these two different phases are reported below.

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Enteral Nutrition During the Early Adaptive Period

The main goal of neonatal nutrition during this phase is to provide immediately the recommended intakes in order to limit the cumulative deficit and to reach a positive nitrogen balance since the first days of life [7, 9, 18]. Despite of many studies enrolling VLBW infants demonstrated that early optimized nutritional support significantly reduces growth restriction during the first days after birth, it might be difficult to satisfy all nutritional needs in this period of life. FI is frequently observed, in the first days of life; thus, PN must be started immediately after birth in all VLBW neonates to maintain adequate fluid, electrolytes, and nutrients intake, until full enteral feeding (120 kcal/kg/d) is reached. In this phase, small amounts of enteral feeding (also called "minimal enteral feeding," "gastrointestinal priming," "trophic feeding," and "hypocaloric feeding") could improve the maturation of the gastrointestinal tract, digestive hormone release, and gut motility. In addition, withholding EN predisposes neonates to the consequences of starvation such as gastrointestinal atrophy, malnutrition, and infections. Minimal enteral feeding (MEF) should be started as soon as possible, when the patient is stable, at a minimal volume of 10–30 ml/kg of body weight, and it could be increased by 20–30 ml/kg of body weight per day if clinical conditions are stable and feeding intolerance does not occur (Fig. 6.1). In the VLBW infant with prenatal history of blood uteroplacental flow alterations, a delayed onset of MEF could be considered [10]. Measurement of flow in mesenteric vessels, by ultrasonography, could be useful in deciding if starting MEF is safe for a neonate [11, 12]. Results of clinical trials in premature infants support the opinion that MEF, preferentially by human milk, has some clinical benefits such as reducing the time to reach full enteral feeding and length of hospitalization without increasing the risk of NEC [11, 12, 19–21]. When signs of FI are not specific nor systemic (Fig. 6.1), MEF could be continued in order to reduce the duration of parenteral nutrition and to limit the risk of infections [12]. MEF should not be considered in the total energy intakes during parenteral nutrition [19].

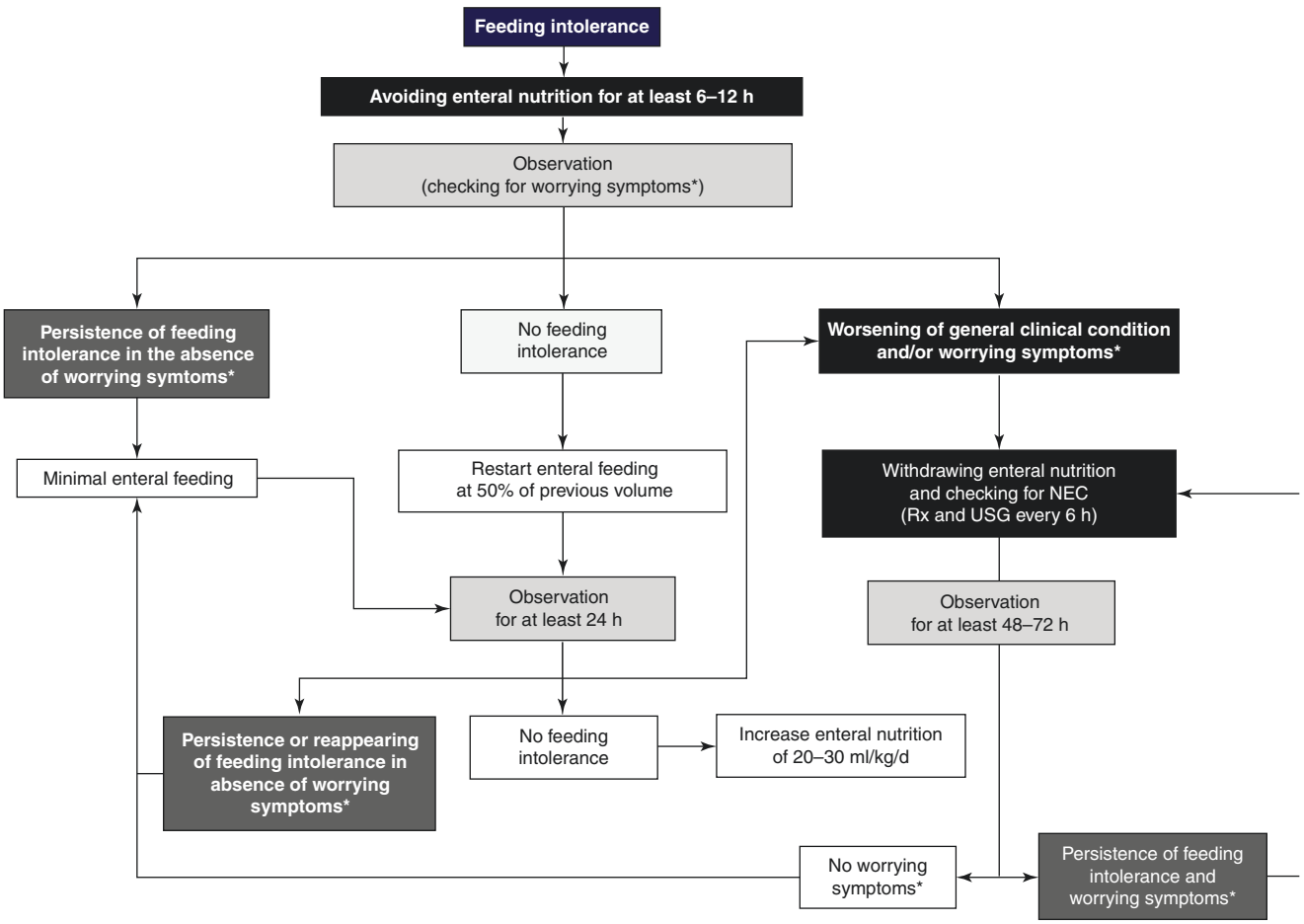

Fig. 6.1 Flow diagram for the management of preterm newborns with feeding intolerance. Note. *Erythematous abdominal wall, absence of bowel sounds, blood in the stools or in aspirates, bile in aspirates, radiological or ultrasonographic markers of NEC Bell stage > I

Table 6.1 Benefits and limits of human milk and formula for preterm neonates

	Nutritional powder	Effects on infectious diseases	Effects on the risk of necrotizing enterocolitis (NEC)
Human milk			
Fresh	Risk of nutrient deficiencies and slower neonatal growth compared to fortified human milk and preterm formula	Reduced risk of infection (sepsis and urinary tract infection) compared to preterm formula feeding Risk of transmission of CMV infection	Reduced risk of NEC compared to preterm formula feeding
Holder pasteurization	Minimal reduction in fat and energy content compared to fresh human milk Partial reduction in nutritional and biological quality compared to fresh human milk	Reduced risk of infection compared to preterm formula feeding. Similar rate of sepsis compared to fresh human milk No risk of transmission of CMV infection compared to fresh human milk	Reduced risk of NEC compared to preterm formula feeding
Short-term heat pasteurization (5 s at 72 °C)	No evidence available	No risk of transmission of CMV infection compared to fresh human milk	No evidence available
Freezing (−20 °C)	Preserve nutritional and biological quality of human milk	Higher risk of transmission of CMV infection compared to holder pasteurized human milk, but reduced compared to fresh human milk	No evidence available
Human fortified milk			
Fortified with bovine-milk based fortifiers	Improvements in growth (weight, length, and head circumference) during hospital stay compared to not-fortified human milk Nutrient availability and properties of human milk may be changed by fortification Lower growth compared to preterm formula feeding Improved growth using target fortification	Lower risk of sepsis compared to preterm formula feeding	Lower risk of NEC compared to preterm formula feeding No significantly increased risk of NEC compared to not-fortified human milk
Fortified with human milk-based fortifiers	Similar rate of growth compared to human milk fortified with bovine-based products	Similar rate of sepsis compared to human milk fortified with bovine-based product	Reduced risk of NEC compared to feeding with preterm formula or human milk fortified with bovine-based product
Preterm formula			
Powdered	Improvement of fat absorption, nitrogen retention, bone mineralization, and growth compared to pooled human milk	Higher risk of sepsis compared to human milk feeding Risk of *Enterobacter sakazakii* infection	Higher risk of NEC compared to human milk feeding
Liquid	Improvement of fat absorption, nitrogen retention, bone mineralization, and growth compared to pooled human milk Reduced bioavailability of various nutrients (proteins, calcium, or copper) compared to powdered formulation due to precipitation or heat treatment	Higher risk of sepsis compared to human milk feeding	Higher risk of NEC compared to human milk feeding

Nutrition During the Intermediate and Stable Growing Period

During this period, parenteral fluid is progressively weaned and stopped when 120 ml/kg/d (100 kcal/kg/d) of EN is well tolerated. EN can be increased up to 140–180 ml/kg per day (115–135 kcal/kg/d), according with the type of formula used and nutritional requirements.

Human milk is the preferred feed for preterm infants in this phase (Table 6.1). Due to the variability of expressed human milk composition, particularly in protein and fat contents, this weaning period from parenteral nutrition is at high risk of relative malnutrition, contributing to cumulative nutritional deficit and postnatal growth restriction [17]. Therefore, it is commonly accepted that human milk (HM) needs to be fortified to meet the nutrient needs of the preterm infant.

It is worth mentioning that transition phase from parenteral to enteral nutrition still remains a critical period for the achievement of adequate growth [19]. It has been demonstrated that the optimization of nutrition during the transition phase, as well as maintaining appropriate nutrient intakes, improves growth rates in preterm infants [19]. However, the lack of nutritional recommendations during weaning from PN complicates the nutritional management of preterm newborns and may contribute to the accretion of nutrients deficit. Thus, it should be paid attention in this phase to limit nutrients deficit, which in turn may result in poor growth rate and altered body composition.

Feeding Modality

Frequency of Feeding: Bolus or (Semi-) Continuous Feeding

Continuous versus intermittent bolus milk feeding in preterm infants has long been a topic of debate. Bolus feeding results in a significant increase in blood flow to the portal-drained viscera (i.e., stomach, spleen, intestine, and pancreas). There is a surge in gastrointestinal hormones, which is higher than when neonates are fed continuously. On the other hand, clinical trials have demonstrated better tolerance with continuous milk feeding in preterm neonates [22]. A significant increase in pulmonary resistance, airflow, and respiratory instability and a decrease in cerebral perfusion have been noted with the bolus feeding method [23]. Several studies have shown that intermittent bolus feedings reduce the time to achieve full enteral feeds, decrease feeding intolerance, and increase weight gain [24]. However, to date literature review is contradicting. A Cochrane review, comparing clinical effects of continuous versus intermittent bolus nasogastric milk feeding in preterm infants, concluded that the present evidence is inadequate for determining an optimal feeding strategy because of the small sample sizes and methodological limitations [25]. Therefore, there is still not enough evidence to recommend either bolus or (semi-) continuous feeding as the preferred method, but the change from one to another can be justified when feeding intolerance persists in smaller neonates.

Oral Feeding

Oral feeding is possible only when the newborn is capable of an adequate suck-swallow reflex. In the term infant, the full, complex, integrated mechanism of swallowing, with the movement of the bolus of milk into the stomach, protection of the airway, inhibition of respiration, and appropriate relaxation of the esophageal sphincter and gastric fundus is achieved within 2 days of life. The swallow function is present from 16 weeks of gestational age, and gastrointestinal motor activity, in small bursts, from 24 weeks onward [26]. Organized motility is present from around 30 weeks of gestation and nutritive and swallowing function from nearly 32 weeks. The very preterm infant (<32 weeks) may be put on the breast but the likelihood that the infant will ingest a significant volume of milk is very small. However, it may offer the mother a considerable psychological benefit and should therefore be strongly encouraged.

Intragastric Feeding

As oral feeding is not possible in VLBW infants, EN is administered through gastric tubes [27]. Either nasogastric or orogastric tubes can be placed; their correct position can be checked (i.e., gastric fundus) by measuring external distance (i.e., distance from nose or mouth to ear plus distance from umbilicus and xiphoid process) or by measuring the pH of aspirate using pH indicator strips/paper. The correct tube placement could be confirmed on a chest x-ray. The nasogastric tube has the advantage of easier fixation but has been noted to increase airway resistance in preterm infants by 30–50%. With the use of some respiratory devices, a permanent nasogastric tube is not possible. An increased incidence of periodic breathing and central apnea has also been noted in association with permanent nasogastric tubes, in preterm infants [27]. Furthermore, persistent tubes may lead to loss of nutrients such as lipids and calcium that may not reach the infant [27]. Hence, the routine use of nasogastric tubes is not advisable for many preterm infants; in this case, intermittent orogastric tube that could be useful for 2–3 consecutive feeds seems to be a more appropriate modality of EN administration.

Transpyloric Feeding

Although transpyloric feeding is frequently used in pediatric intensive care, no proven benefits in tolerance, growth, or in aspiration rates have been reported in neonates. In addition, there is rising evidence suggesting an increase in NEC and in mortality rate in neonates receiving transpyloric feeding [27].

Feeding Tolerance Evaluation and Feeding Advancement

FI may sometimes be obvious when severe gastrointestinal manifestations are identified (emesis, vomiting, severe abdominal distension, ileus with visible intestinal loops, and

blood in the stools) or when gastrointestinal symptoms are associated with systemic disorders like apnea, bradycardia, poor perfusion, and/or hemodynamic instabilities. However, intermediate scenarios are frequent in preterm infants. Routine monitoring of gastric residual in preterm infants on gavage feeds is a common practice, which is used to guide initiation and advancement of feeds [20]. Gastric residual volume measurement is the practice by which nurses try to aspirate (suck out) the whole of the child or infant's stomach contents every few hours to assess the volume and appearance of the stomach contents [20]. The aspiration of gastric residuals is a common practice while tube feeding infants and aspirate usually need to be reinfused to reduce the loss of enzymes and electrolytes. Routine monitoring of gastric residual as a guide, in the absence of uniform standards, may lead to unnecessary delay in initiation and advancement of feeds and delay in reaching full enteral feeds. This in turn may increase the delay in achieving full enteral feeds, the risk of extrauterine growth restriction, and neurodevelopmental impairment. However, a number of literature suggests that an increase in/or an altered gastric residual may be predictive of NEC [15, 16, 21]. Withholding monitoring of gastric residual may take away the early indicator and thus may increase the risk of NEC.

Thus, the strategy of not checking routinely gastric residual volumes should be adopted only after an appropriate training of the caregivers. When stomach residual volumes are used as markers of FI, it should be appropriate to establish reference values. Gastric residuals >4 ml/kg or >50% of 3 h previous feed could be considered as a valid criterion to interrupt or reduce enteral feeding [20, 21, 28]. In the absence of signs of FI, the volume of enteral nutrition could be daily increased. The ideal rate of progression of milk volume given to VLBW infants is controversial. Cochrane Review has concluded that a rapid rate of advancing feeds was not associated with a higher risk of NEC [20]. Although additional studies are needed, a small progressive increment of 20 ml/kg per day after a few days of stabilization can be recommended (Fig. 6.1). In the presence of erythematic abdominal wall, absence of bowel sounds, blood in the stools or in aspirates, enteral nutrition should be discontinued and serial abdominal ultrasounds and radiological examinations should be started in order to early identify NEC [15, 16, 20, 21, 28].

Nutrient Needs During Enteral Feeding

Protein

Protein Requirements

The ideal amount of dietary protein for preterm infant is still a matter of debate. Dietary protein needs for preterm infants are calculated through two different methods [18, 29]. The first one, the empirical approach, measures biochemical or physiological responses to graded intakes. The second method, the factorial approach, considers the requirements as the sum of the essential losses (e.g., urine, feces, skin), plus the amount incorporated into newly formed tissues, accounting theoretical fetal accretion at the same PMA. The empirical approach evaluates physiological and biochemical variables to determine minimum and maximum protein needs in the growing preterm infant.

In the factorial approach, compositional analysis of fetal tissues has been a valuable source of data for our understanding of the nutrient needs of the fetus and, by extension, those of the growing preterm infant. Fetal accretion rates have been obtained from compositional analyses of aborted fetuses or stillborn infants. From these data, the protein increment for growth has been estimated as approximately 2.5 g/kg/d [29]. Protein requirement is thus calculated by adding to this value the obligatory losses, and the amount needed for the additional catch-up growth. In a large population of preterm infants receiving a controlled energy intake, the minimal protein supply necessary to obtain a zero nitrogen balance (to cover all nitrogen losses) was calculated to be 2 g/kg/d. A similar value was estimated to cover the need for catch-up growth. Thus, the protein requirements of premature infants are between 3.5 and 4.5 g/kg/d (Fig. 6.2) [29].

Protein intake must be sufficient to achieve normal growth without negative effects such as acidosis, uremia, and elevated levels of circulating amino acids [18, 31]. A recent Cochrane analysis aimed to determine whether higher (≥3.0 g/kg/d) versus lower (<3.0 g/kg/d) protein intake during the initial hospital stay of formula-fed preterm infants or low birth weight infants (<2500 g) results in improved growth and neurodevelopmental outcomes without evidence of short- and long-term morbidity [32]. Five studies compar-

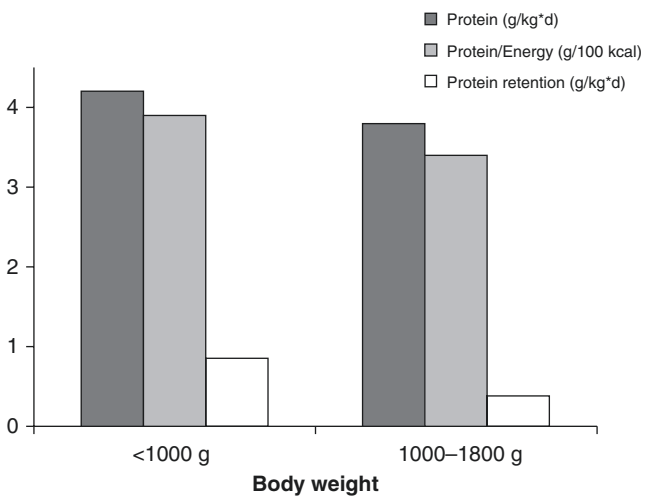

Fig. 6.2 Recommended protein intake and protein to energy ratio for preterm infants to obtain optimal protein retention [9, 30]

ing low versus high protein intake were included. Improved weight gain and higher nitrogen accretion were demonstrated in infants receiving formula with higher protein content while other nutrients were kept constant. No significant differences were seen in rates of NEC, sepsis, or diarrhea. One study compared high (3 g/kg/d) versus very high (>4 g/kg/d) protein intake during and after an initial hospital stay [33, 34]. Very high protein intake improved gain in length at term, but differences did not remain significant at 12 weeks corrected age. Three of the 24 infants receiving very high protein intake developed uremia, defined as serum urea nitrogen level greater than 6. Formula with higher protein content improves all growth parameters and neurodevelopment [35, 36]. However, available evidence is not adequate to permit specific recommendations regarding the provision of very high protein intake (>4.5 g/kg/d) from formula during the initial hospital stay or after discharge.

Enteral Nutrition Protein Composition

Nitrogen absorption rate (absorbed/intake) differs significantly according to the feeding regimen (Fig. 6.3). It is higher with powder whey-predominant protein preterm formulas than with human milk supplemented with fortifiers, powder protein-hydrolyzed formulas, or ready-to-use liquid whey-predominant preterm formulas. These differences result from the nature of the various types of protein supply. Also net protein utilization is higher for preterm formulas compared with human milk, with or without protein supplementation. Technical processes (i.e., hydrolysis) may alter macronutrient concentrations of formula, and the pasteurization of human milk before delivery to newborn also cause a reduction in the protein concentration of about 4% [37, 38].

The whey/casein ratio significantly influences individual amino acid intakes and plasma amino acid concentrations. Plasma threonine is increased and tryptophan relatively decreased in infants fed a whey-predominant formula, whereas methionine and aromatic amino acids are increased in those fed a casein-predominant formula [38]. The high plasma threonine concentration observed in preterm infants fed whey-predominant formulas is related to the glycomacropeptide obtained from casein by enzymatic casein precipitation of cow's milk proteins. Acidic precipitation, by contrast, removes the glycomacropeptide rich in threonine from the soluble phase [38]. In preterm infants receiving either an enzymatic or an acidic whey-predominant formula, a significant reduction in plasma threonine concentration was observed in acidic whey protein formula-fed infants, compared to those receiving conventional enzymatic whey protein formulas. All other plasma amino acid concentrations were similar, with the exception of valine, which was slightly reduced in acidic whey protein formula-fed infants [30].

The relative percentage of α-lactalbumin depends on the ratio between different cow's milk protein contents [30]. Indeed, this protein fraction is naturally rich in tryptophan and helps normalize the low levels of this amino acid frequently observed in whey-predominant formula-fed infants. Formulas based on hydrolyzed proteins have been recently proposed for the feeding of preterm infants to reduce gastrointestinal problems such as delayed gastric emptying, abdominal distension, hard stools, and feeding intolerance [39]. The technological processes necessary to perform hydrolysis and reduce protein antigenicity may, however, modify the amino acid content and/or amino acid bioavailability [39]. The use of a higher percentage of whey in protein-hydrolyzed formulas may worsen the plasma amino

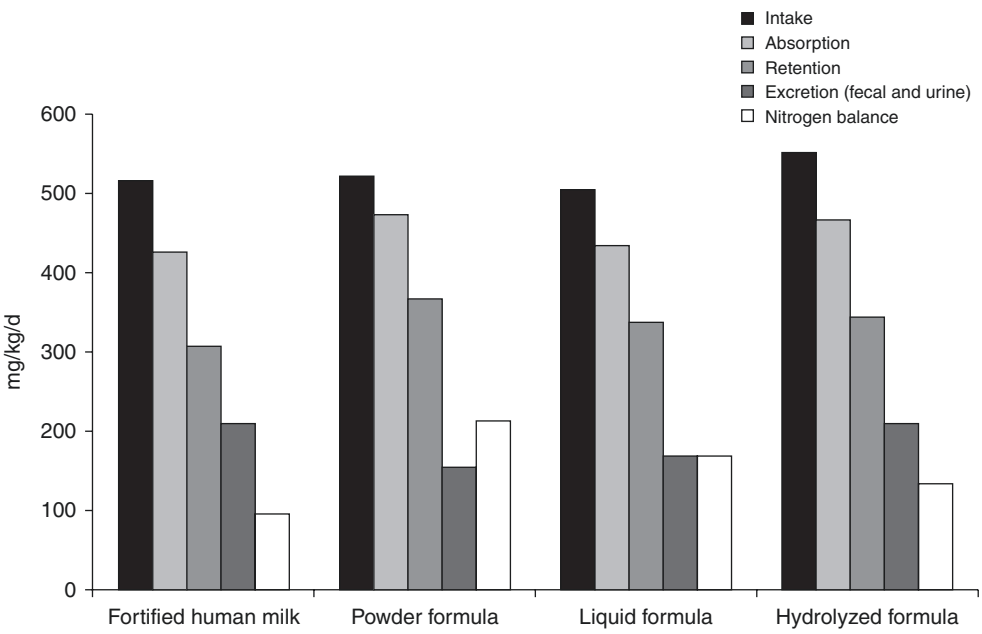

Fig. 6.3 Nitrogen balance according to feeding regimens in preterm infants [27]

acid pattern by increasing threonine and decreasing aromatic amino acid concentrations [38]. Moreover, a significant decrease in plasma histidine and tryptophan concentrations, probably due to a relative reduction in amino acid bioavailability, could also be observed. Using a more appropriate technology, these formulas have been corrected for the threonine content and supplemented with histidine and tryptophan [40, 41].

An interesting observation is the relatively lower absorption rate of ready-to-use liquid preterm formulas or protein-hydrolyzed preterm formulas, where the technical process seems to impair nitrogen absorption in relation to heat treatment—inducing some Maillard reaction—or to preliminary hydrolysis, altering the physiological absorption process in the lumen or at the border of the gastrointestinal tract. The efficiency of protein gain, estimated by the ratio between retained and metabolizable nitrogen (absorbed), differs also according to the feeding regimen. The highest values were obtained in preterm infants fed powder and liquid preterm formulas. It was significantly lower in those fed protein-hydrolyzed formulas and fortified human milk. The lower value obtained with fortified human milk may be related to non-protein nitrogen content, which represents 20–25% of total nitrogen content of human milk, but still 13.5–17% of total nitrogen content of fortified human milk. The contribution of non-protein nitrogen fraction of fortified human milk to protein gain is lower than that of the α-lactalbumin or the casein content of human milk [30, 36, 38, 41]. Due to its biological activities, the non-protein nitrogen of human milk (HM) may not be included in protein intakes when HM is compared to formulas. This may explain why we still frequently observe differences in growth rate of infants fed HM or PTF with theoretical similar nitrogen content. On the other hand, formulas do not contain any of the biologically active immune substances, nor enzymes, hormones, or growth factors found in HM [31–33]. The long-term implications of this deficiency have not been completely determined. Further long-term randomized studies with higher number of patients and standardized supplemental amounts and experimental conditions are needed to establish the efficacy of non-nutritional compounds, such as nucleotides, polyamines, and growth factors supplementation in preterm formula [18].

Energy

The energy demands for preterm infants depend on many factors including PMA, genetically determined metabolic rate, thermal environment, activity, sleep status, nutritional status, nutrient intake, body composition, and occurrence of illness [41, 42]. Energy expenditure, measured by indirect calorimetry, increases with postnatal age and varies from 45 to 55 kcal/kg/d. The energy cost for a postnatal weight gain of 17–20 g/kg/d with adequate LBM accretion varies from 50 to 70 kcal/kg/d in premature infants [41–43]. Energy absorption from enteral nutrition, in healthy growing premature neonates after the first 2–3 days of life, is about 80–90% of energy intake. The remainder is lost in stool and a small quantity as urea in the urine. Energy absorbed is in part stored in tissues, mainly as fat but also as protein, and in part spent by the metabolism (energy expenditure). Energy intakes less than or equal to 100 kcal/kg/d will not meet the needs of some preterm infants before discharge. Where PER are adequate (3.2–3.6 g/100 kcal) in a formula providing well-absorbed nutrients, an energy intake around 120 kcal/kg/d is generally appropriate and may result in a fat mass deposition close to intrauterine references. Small-for-gestational-age infants, in particular if they experienced intrauterine malnutrition, may need higher energy intake than appropriate-for-gestational-age infants [8, 17, 41–43]. Energy intake recommended for preterm infants is 115–135 kcal/kg/d [8, 42, 43]. All recommendations are related to preterm formula, but energy requirement could differ in preterm receiving HM by contrast to those fed with preterm formula [8, 41–44].

Protein to Energy Ratio

Protein intake and PER are the main determinants of weight gain [33]. Protein intake is the only determinant of lean body mass gain in contrast to fat mass gain, which is positively related to energy intake and negatively to PER. Protein and energy needs are reciprocally limiting, the intake of one affecting the ability of the infant to assimilate the other [33, 43, 45]. A suboptimal range of the PER leads to untoward consequences. If energy intake is inadequate, proteins are used as an energy source and the nitrogen balance becomes less positive. Increasing the caloric intake will spare protein loss and improve nitrogen retention. If there is a surfeit of energy with limited protein intake, the protein retention reaches a plateau and the energy excess is used for excessive fat deposition. Thus, an adequate PER should be respected at all PMA, with adequate partition of energy intake between fat and carbohydrates. This crucial point should be respected particularly in the period of clinical instability, when nutritional regimens in a significant part depend on parenteral nutrition [45]. Recommended intakes for preterm infants at 26–30 weeks' PMA should be 3.8–4.5 g of protein/kg per day with a high PER above 3.2 g/100 kcal and ideally around 3.6 g/100 kcal [8]. A recent experts panel has recommended 3.6–4.1 g/100 kcal in VLBW infants, while the ESPGHAN have recommended 3.6–4.1 g/100 kcal in ELBW infants and 3.2–3.6 g/100 kcal for other preterm infants <1800 g [8, 45].

Fat

Fat Requirements

Fat provides the major source of energy in growing preterm infants. Recommended fat intake is 4.8–6.6 g/kg/d [8]. Although some infants with restricted fluid may need high fat content in their feeds to meet energy needs, 4.4–6.0 g/100 kcal is a reasonable range for most preterm infants [45]. Lipid availability by enteral route depends more on digestion and absorption capability than on enteral nutrition content and composition. Digestion of lipids is not fully developed in premature infants [5]. Lipid malabsorption is the result of low levels of pancreatic lipase and bile salts. The bile acid pool is only half the size of that of a full-term infant. HM provides additional lipases: lipoprotein lipase, bile salt-stimulated lipase, which improves intestinal lipolysis. However, additional fat should also be provided to HM because of the variability of fat content in expressed HM, especially when various processes are applied to HM prior to delivery to neonates [45, 46].

Enteral Nutrition Fat Composition

Fats represent approximately 50% of the non-protein energy content of human or formula milk. Fat could be provided in different forms with different nutritional power.

Medium-chain triglycerides (MCT) MCTs are hydrolyzed more readily than long-chain triglycerides (LCT), and fatty acids with more double bonds are absorbed more efficiently [45, 47, 48]. In order to improve fat absorption, commercial formulas contain a significant quantity of MCTs that are more easily absorbed than LCTs and transported directly to the liver via the portal vein as nonesterified fatty acids. The use of MCTs instead of LCTs in preterm infant formulas can reduce the formation of calcium and magnesium soaps with unabsorbed long-chain saturated fatty acids and, thereby, increase calcium and magnesium absorption. However, it needs to be stressed that the energy content of MCTs corresponds only to 85% of that of LCTs. The use of MCTs in formulas induces an increase in plasma ketones as well as in urinary excretion of dicarboxylic acids, suggesting that MCT metabolism could be slightly limited [45, 47]. Moreover, since MCTs do not contain essential fatty acids, a high MCT intake can reduce the availability of the essential long-chain fatty acids. For these reasons, it is recommended that the maximum MCT intake in preterm formula be limited to 40% of total fat content [8, 45].

Long-chain Triglycerides (LCT) Because humans cannot insert double bonds at the n-3 and n-6 positions, fatty acids with double bonds in these positions cannot be synthesized endogenously. Therefore, either specific n-3 and n-6 fatty acids or the precursor of each series such as linoleic acid (LA) (18:3n-3) and α-linolenic acid (ALA) (18:2n-6) must be provided as a component of the diet. In preterm infants, LA intakes of 3.2–12.8% of energy intake have been recommended [8, 48, 49]. Both LA and ALA are metabolized by a series of desaturation and elongation reactions to more unsaturated longer-chain fatty acids. Important metabolites of these two fatty acids include 20:5n-3 eicosapentaenoic acid (EPA), 22:6n-3 docosahexaenoic acid (DHA), and 20:4n-6 arachidonic acid (AA) [47, 48].

Recent studies have reported outcome data in preterm infants fed milk with a DHA content 2–3 times higher than the current concentration in infant formulas. Overall, these studies show that providing larger amounts of DHA supplements, especially to the smallest infants, is associated with better neurologic outcomes in early life [47]. DHA intakes of 55–60 mg/kg/d from the time of preterm birth to expected term have been tested and appear to be safe, to promote normal DHA status, and to improve visual and neurocognitive functions. These values are likely to represent an adequate intake for very preterm infants, but further research is needed to confirm that they represent adequate intake for all infant groups (i.e., extremely, very, and moderately preterm infants, with or without intrauterine growth restriction, and males and females). Because the maximum DHA content of HM is >1.5% of fatty acids (equivalent to 84 mg/kg/d), and because no studies with such a high intake in preterm infants have been reported, no upper limit can be set with certainty.

Considering that endogenous synthesis of long-chain polyunsaturated fatty acids (LCPUFAs) is limited in preterm infants, these molecules are considered as semi-essential or essential. Several studies have suggested that the LCPUFAs supply in preterm infants has a beneficial effect on growth, visual, and cognitive function, as well as on the immune system. Although some methodological issues in these studies do not allow definitive conclusions to be drawn, most of the formulas in Europe and the USA are currently supplemented with LCPUFAs [45, 48, 49].

Recommended intakes are 12–30 mg/kg/d or 11–27 mg/100 kcal for DHA and 18–42 mg/kg/d or 16–39 mg/100 kcal for AA [8]. The ratio of AA to DHA should be in the range of 1.0–2.0 to 1 (wt/wt), and EPA supply should not exceed 30% of DHA supply [8].

Fat composition of HM While the amount and composition of carbohydrate and protein remain relatively constant in mature HM, the composition of fat is highly variable and is affected within hours and to a large extent by maternal nutrition intake. Gestation, lactation, parity, milk volume, caloric and carbohydrate intake, and weight changes are among the maternal factors that can alter the fat content and composition of breast milk. Specifically, phospholipid and cholesterol content are higher in colostrum preterm than term breast milk. In addition, LCPUFA are higher in preterm

and transitional milk and remain high for the first 6 months in women who deliver preterm. In term milk, on the other hand, LCPUFA declines throughout the first 6 to 12 months of lactation. Fatty acids rise with a high-carbohydrate maternal diet. Palmitic acid content of breast milk increases in a low-calorie diet. Weight gain during pregnancy is positively associated with higher milk fat content. During infant feedings, foremilk has less fat content than hindmilk. Moreover, the higher the volume of breast milk, the lower the milk fat concentration. The lengths of both gestation and lactation affect the lipids that constitute the milk fat globule membrane. Total phospholipid and cholesterol level is higher in colostrum and transitional milk than in mature milk. The period of colostrum lasts less than 10 days, but during this short time the higher lipid levels are beneficial in such processes as neonatal cell membrane production needed for growth, brain development, and bile salt synthesis. LCPUFA levels normally decrease in breast milk during lactation, but in women who have delivered infants before term, the levels remain constant in preterm milk for at least 6 months. Milk fat content changes during each feeding. There is a strong positive relationship between weight gain during pregnancy and milk fat content. Preterm milk may contain a slightly higher proportion of medium- and intermediate-chain fatty acids than term milk, which may be advantageous for fat and calcium absorption in preterm infants. The estimated mean fat content in banked HM is around 3.2 g/100 ml, somewhat lower than the generally accepted value for mature HM [46, 50, 51]. This may be related to inadequate emptying of the breast during pumping or to processes applied before delivery DHM to neonates.

Pasteurization and storage of DHM induces lipolysis, inactivates bile salt-stimulated lipase and lipoprotein lipase, reduces fats, and increases the absolute amount of free fatty acids in pooled samples. Holder pasteurization involves a modification of the biological activity of a large number of enzymes, including lipases. These effects alter the integrity of HM and may contribute to slower growth in preterm infants fed banked milk versus their mothers' own milk. Based on this amount of evidence, a further supplementation with specific fatty acids could be considered for these infants [45, 50, 51].

Carbohydrates

Carbohydrate Requirements

Carbohydrates are essential to provide adequate energy supply and they represent a major source of dietary energy. Based on carbohydrate equivalents of total energy expenditure, the experts have recommended a minimum of 10.5 g/100 kcal and a maximum of 12.0 g/100 kcal [8, 45]. Carbohydrates are essential to provide adequate energy supply. Additionally, carbohydrates promote epithelial cell proliferation, insulin secretion, and calcium absorption [8, 45]. Thus, they are also essential to the overall health of the gastrointestinal tract other than the fulfillment of energy requirements.

Enteral Nutrition Carbohydrate Composition

The predominant carbohydrate in mammalian milk and in term infant formula is lactose. After digestion by lactase, lactose is absorbed as glucose and galactose, which utilize the same carrier mechanism. In the human fetus, intestinal lactase activity is measurable by 10–12 weeks of gestation [52]. There is a gradual increase in lactase activity with advancing gestation, although the activity remains low until about 36 weeks of gestation, when it reaches the levels seen in full-term neonates [52]. Based on the low lactase activity in early gestation and the estimated length of the bowel, it was calculated that a preterm infant weighing 1300–1400 g might be expected to absorb only 30–50% of the ingested lactose. The lactose that is not fully digested serves as a source of nutrition for bacterial flora in the colon, where it is transformed into short fatty acids and then absorbed [5]. Through the process of fermentation, this not only facilitates colonic water and electrolytes absorption but also stimulates cell turnover of both the colon and the small intestine. In addition to lactose, which serves as an energy source, human breast milk contains 7–25 grams of complex sugar molecules (oligosaccharides) per liter. These various sugars are only found at significant concentrations in HM, and they are collectively known as HM oligosaccharides (HMOs). Oligosaccharides represent the third largest solute load of the diet after lactose and fat [52]. Oligosaccharides are hydrolyzed by salivary, pancreatic, and intestinal amylase and maltase to free glucose, which is rapidly absorbed. Many of the protective effects of HM (i.e., against diarrheal diseases, respiratory, and ear infections) have been attributed to oligosaccharides. A further advantage of oligosaccharides for preterm infant formulas includes the improved gastric emptying [52, 53]. Oligosaccharides may be considered as prebiotics. There are some reports that such prebiotics have beneficial effects on various markers of health. For example, primary prevention trials in infants have provided promising data on prevention of infections and atopic dermatitis. However, additional well-designed prospective clinical trials and mechanistic studies are needed to advance knowledge further in this promising field.

Fluids and Electrolytes

The goal of fluid administration is to replace water loss, maintain water and electrolyte homeostasis to provide extra water and electrolytes to build up new tissues. Total fluid

intake is related to ingested caloric and protein intake as well as to the renal solute load [54, 55]. Randomized controlled trials on enteral fluid intake of preterm infants are lacking as are studies comparing different fluid volumes providing identical nutrient intakes. From data of combined parenteral/enteral regimens, and assuming full enteral absorption, it follows that fluid volumes between 96 and 200 ml/kg/d are tolerated, and that these values may serve as lower and upper limits [8]. When exclusively enteral feeding is reached, a rate of 150–180 ml/kg/d by standard formula or fortified breast milk should be provided to meet nutrient requirements. Some infants may need higher volumes to meet higher requirements of substrates other than fluid. Preterm infants of less than 35 weeks gestation have obligate high renal and intestinal sodium losses during the first 2 weeks of life, leading to cumulative negative sodium balance in most and hyponatremia in many. It has been demonstrated that increasing dietary sodium intake to 4 mmol/kg/d for infants born at 31–34 weeks, and to 5 mmol/kg/d for those born before 31 completed weeks, prevents the negative sodium balance and hyponatremia and leads to more rapid weight gain and earlier discharge from hospital [54–56]. Premature infants require a higher sodium intake in the first 2 weeks of postnatal life than those born at or near term, and failure to provide such an intake may predispose to poor neurodevelopmental outcome in the second decade of life [57]. Potassium intakes are estimated around 2–3 mmol/kg [8, 58].

Calcium and Phosphorus

Calcium and Phosphorus Requirements

After birth, the use of the gastrointestinal tract to provide all nutrients for growth causes a large reduction in calcium bioavailability [59–61]. Various factors affect calcium absorption: vitamin D status, solubility of calcium salts, and the quality and quantity of fat intake. In preterm infants, the vitamin D body stores at birth depend mainly on maternal vitamin D status. In the USA, where dairy products are routinely supplemented with vitamin D, a daily additional intake of 400 IU in the formula may sometimes be sufficient to maintain an adequate plasma concentration of 25-OH and 1–25(OH)2 vitamin D. By contrast, in most parts of Europe, cord blood concentration of 25-OH vitamin D of premature infants is frequently less than 10 μg/ml; in this case, up to 1000 IU/d of vitamin D are recommended [8, 60]. The increased needs of premature infants could be partly due to a relative malabsorption of vitamin D, resulting from a low secretion of bile acids [62]. A calcium retention close to 90 mg/kg per day could currently be expected in preterm infants fed preterm formula with a highly soluble calcium content. These values are still lower than the estimated fetal accretion during the last trimester of gestation (100–120 mg/kg per day). However, there are dramatic physiological changes in bone metabolism resulting from various factors after birth: disruption in maternal mineral supply, stimulation of calciotropic hormone secretion, change in hormonal environment, and relative reduction in mechanical stress. These events stimulate the remodeling process leading to an increase in endosteal bone resorption and a decrease in bone density. In preterm infants, these adaptation processes modify the mineral requirement, since, by itself, the increased remodeling and bone turnover provides a part of the mineral requirement necessary for postnatal bone growth [55, 60].

Phosphate is one of the main intracellular ions present in the cytoplasm. The provision of adequate amounts of nitrogen, potassium, and phosphorus is the condition required to obtain adequate lean body mass accretion and rapid cell growth. The phosphorus to nitrogen ratio in the cells is not stable, and it is increased in the rapidly growing tissues. A large amount of phosphorus is also deposited in the bone in a fixed proportion with calcium and can act as mineral reservoir. In fact, regardless of the proper bone metabolic status, phosphorus consumption by the cell metabolism is privileged in the growing newborn, and phosphorus may be released into circulation from the bone if necessary for cellular requirements. In case of insufficient phosphorus intakes, hypophosphatemia occurs due to cell metabolism requirements. Afterward, and due to bone phosphorus mobilization from the bone, bone calcium release induces hypercalcemia and hypercalciuria [60]. The strict relationship among amino acid, calcium, and phosphorus intakes is not specific to the first days of life of parenterally fed neonates. This phenomenon of phosphorus deprivation may also be observed in preterm infants enterally fed when a supplementation of protein was not accompanied by a modification of the Ca/P ratio. Calcium retention level ranging from 60 to 90 mg/kg/d assures appropriate mineralization, it decreases the risk of fracture, and it diminishes the clinical symptoms of osteopenia. An intake of 100–160 mg/kg/d of highly bioavailable calcium salts, 60–90 mg/kg/d of phosphorus, and 800–1000 IU of vitamin D per day is recommended [8].

Calcium and Phosphorus Composition in Enteral Nutrition

In preterm infants fed HM, calcium absorption range from 60% to 70% depending on the calcium salts and intake. Calcium retention is mainly related to phosphorus supply, which is frequently the limiting factor of bone mineralization. Supplementation of HM with phosphorus alone improves calcium retention from 25 to 35 mg/kg/d. When calcium and phosphorus are provided together or as HM fortifiers, calcium retention may reach 60 mg/kg/d. Recent use of HM fortifiers containing highly soluble calcium glycerophosphate has improved calcium retention up to 90 mg/kg per day at intake of 140 mg/kg/d [8, 60]. In formula-fed

infants, calcium absorption is usually less than with HM, ranging from 35% to 60% of intake. Calcium absorption is related to calcium, fat intakes, and techniques of milk preparation [8]. With ready-to-use liquid formulas, calcium absorption is usually lower than with powder formulas. With the use of formulas with a well-absorbed fat blend of about 85%, the formation of calcium soap is of minimal interest in clinical practice. Finally, owing to the poor solubility of calcium salts, especially calcium phosphate, the calcium content measured in the formula could be significantly lower than the claimed value, and additional loss due to precipitation may occur before feeding.

Iron

Preterm infants require iron for erythropoiesis, brain development, muscle function, and cardiac function. The symptoms of iron deficiency are not due to anemia only but are also due to tissue losses of iron-containing enzymes and iron–sulfur proteins. It has been estimated that, in the absence of iron supplementation, a VLBW preterm infant has enough iron stores to last 2 months [63]. However, due to their growth requirements and blood samples, preterm infants need supplemental iron after 2 weeks of age. Enteral dose of iron for the preterm infant ranges from 2 to 4 mg/kg/d, depending on the degree of prematurity and the amount of phlebotomy. The introduction of erythropoietin puts a greater stress on iron balance, forcing the infant to mobilize endogenous iron stores at a faster rate. For infants receiving this medication, an enteral dose of 6 mg/kg/d is recommended. No study has demonstrated oxidative toxicity from enteral iron given at conventional doses in preterm infants. Iron needs of preterm infants remain greater than those of term infants because of their more rapid relative rate of growth and therefore blood volume expansion. Given their lower endogenous iron stores, it would be prudent in preterm to monitor the presence of anemia earlier than in term infants.

Trace Elements

Trace elements contribute less than 0.01% of total body weight. Functionally, they participate as constituents of metalloenzymes, cofactors for metal ion-activated enzymes, or components of vitamins, hormones, and proteins. Preterm neonates are at particular risk to develop trace elements deficiency due to low body stores because of reduced time for their placental transfer, which normally occurs during the third trimester of pregnancy [64, 65]. Trace minerals with established physiological importance in humans include zinc, copper, selenium, manganese, chromium, molybdenum, and iodine. In particular, zinc has been shown to be crucial for ensuring adequate growth. Zinc is, indeed, a key element for protein synthesis in preterm newborns. Zinc requirements for term infants are estimated to be 0.8 mg/d, whereas preterm neonates may require up to 3 mg/kg/d to achieve adequate zinc retention [64]. Zinc content in HM varies considerably (0.7–1.6 mg/L) and declines with time; while colostrum contains 8–12 mg/L, HM at 7 days of neonatal life contains 3–6 mg/L of zinc. These values rapidly decrease at 1–3 mg/L at 1 month of life [64, 65]. Recently, it has been showed that doses of zinc higher than those recommended by the ESPGHAN may reduce morbidity and mortality in preterm neonates [66]. Furthermore, it has been suggested that early aggressive nutrition in preterm newborns, promoting protein synthesis, may cause electrolyte imbalances, especially it may increase zinc consumption [67]. The current nutritional practices for preterm neonates consist of administering high doses of protein and calories as soon as possible after birth. Zinc is required for the activity of a number of proteins (i.e., enzymes, membrane proteins, gene-regulatory proteins, and hormonal receptors), it has a crucial role in maintaining quaternary structure stability of proteins, and it participates in the regulation of gene expression, cell division, and growth [8, 64, 65]. Consequently, during anabolic processes which result in higher protein synthesis, particularly after birth, the requirements of zinc increase. The recommended enteral intake ranges between 0.8 and 3 mg/kg/d [64, 65, 67]. For these reasons, an increased protein and energy intake should be associated with an increased zinc intake.

Oral Vitamin Requirements

Vitamins are organic compounds that are essential for metabolic reactions but are not synthesized by the body [8, 45]. Vitamins are classified as water soluble or fat soluble, based on the biochemical structure and function of the compound. Water-soluble vitamins cannot be formed by precursors (with the exception of niacin from tryptophan) and do not accumulate in the body (with the exception of vitamin B12). They include B complex vitamins and vitamin C. They serve as prosthetic groups for enzymes involved in amino acid metabolism, energy production, and nucleic acid synthesis. A daily intake is required to prevent deficiency. Excretion occurs in the urine and bile. Altered urinary losses due to renal immaturity during the first week of life predispose a preterm infant to vitamin deficiency or excess [8]. Fat-soluble vitamins include vitamins A, D, E, and K. Fat-soluble vitamins require carrier systems, usually lipoproteins, for solubility in blood, and intestinal absorption depends on fat absorption capability [8]. Vitamins are not required daily and deficient states develop slowly. On the other hand, excess of vitamins may produce toxicity.

Human Milk

Benefits of Human Milk

Since 20 years, the American Academy of Pediatrics (AAP) has acknowledged the advantages of HM feeding with the statement that it is the preferred feeding for all infants, including those born preterm [68]. However, not all mothers can provide sufficient milk to meet requirements; thus, supplementation with either preterm formula (PTF) or donor human milk (DHM) is a common practice. Current recommendations are for the use of mother's own milk (MOM), when available, with appropriately screened and pasteurized DHM the next best choice if there is insufficient MOM [68, 69]. Randomized trials (RT) and meta-analyses provide evidence of the relative advantages of HM feeding compared with formula feeding [68, 69]. Advantages of HM feeding, with either pasteurized DHM or MOM, include protection from NEC, infection, retinopathy of prematurity (ROP), and improved cognitive outcomes [68, 69].

Cohort studies, comparing infants fed any HM with infants fed EPTF, demonstrated a clear effect of any HM in reducing NEC. However, the evidence of positive effect of HM was inconclusive for severe NEC. Preterm infants fed HM have fewer infections than those fed on formula. This advantage has been observed with both fresh and pasteurized HM [68]. Recent meta-analysis on the effect of HM on ROP showed a protective effect of HM on ROP and severe ROP for both MOM and DHM in comparison with preterm formula. The protective effect of HM has been attributed to several factors, such as macrophages, lymphocytes, sIgA, lysozyme, lactoferrin, oligosaccharides, nucleotides, cytokines, growth factors, and enzymes present in breast milk. Immune defense can be provided by an interaction between these factors. Breast milk enhances the growth, motility, and maturity of the gastrointestinal tract, compared to formulas. It also induces faster gastric emptying. The mother's presence in the neonatal nursery combined with breast milk expression, particularly the premature infant's skin-to-skin contact with the mother, may reduce the risk of infections by nosocomial pathogens by passive immunization [68, 69]. Preterm infants fed HM during hospitalization showed better neurological development and a higher intelligence quotient compared to those fed formula, even after controlling for the mother's education and social class [68, 69]. Higher mental developmental index scores were reported in VLBW infants at 18 months of age who received HM, compared to those who never received HM in hospital [69]. At 16 years follow-up, the percentage of expressed breast milk in the neonatal diet correlated significantly with verbal IQ and white-matter volume in males. Potential factors of HM that might contribute to neurodevelopment are net protein utilization, amino acid and fat composition, other than hormone, growth factor, and micronutrients [8].

Limits of Human Milk in Preterm Newborns

Despite many benefits, HM and its nutrients content are not sufficient to cover the greater needs of VLBW infants. Breast milk composition depends on the gestational age at delivery, collection methods (e.g., drip method versus expression with a pump), and whether pooled milk or the infant's own mother's milk is used [69]. Greater concentrations of nitrogen, immune proteins, total lipids, medium-chain fatty acids, energy, vitamins, some minerals (calcium and phosphorus), and trace elements in milk from mothers who give birth prematurely (preterm milk) compared with milk of mothers giving birth at term (term milk) have been observed [68]. The higher concentrations of these nutrients tend to decline as lactation progresses, so exclusive feeding of mature preterm milk from 2 weeks postnatally may lead to nutrient deficiencies in the rapidly growing preterm infant [50]. The reason for the difference in nutrient density between preterm and term milk is not well known. Early interruption of pregnancy might induce an incomplete maturation of mammary glands leading to paracellular leakage of serum proteins and ions through junctions that have not completely closed. In addition, a different hormonal profile in women who deliver prematurely compared with those who deliver at term could be responsible for a different milk composition. The greater nutrient density in preterm milk could also be related to the higher concentration of nutrients in a lower volume of milk. One of the main limits of HM for preterm infants is its production. Mothers of preterm infants, for several reasons (stress, poor maternal health, delayed initiation of lactation), frequently produce an insufficient quantity of milk. The difficulties faced by mothers trying to provide milk for their preterm infant must not be underestimated. They may need to express milk for a period of weeks or months in the absence of significant suckling stimulus and in the presence of a great deal stress. Increasing the frequency of expression, kangaroo care, or skin-to-skin contact, relaxation tapes may improve milk volume produced by mothers. Moreover, a restriction of fluid intake in VLBW infants could reduce the volume of HM given, and thus not offer a sufficient quantity of nutrients.

Human Milk Fortification

HM does not provide sufficient nutrition for VLBW infant when fed at the usual feeding volumes leading to slow growth with the risk of neurocognitive impairment and other poor

health outcomes such as retinopathy and bronchopulmonary dysplasia. HM should be supplemented (fortified) with the nutrients in short supply, particularly with protein, calcium, and phosphate, to meet the high requirements of this group of babies.

The protein content of HM is too low to permit a weight gain similar to the intrauterine fetus. The low sodium level may lead to hyponatremia. The amounts of calcium and phosphorus is widely below the intake needed to achieve adequate bone mineral accretion and to avoid severe osteopenia.

Fortified human milk feeding regime significantly improves growth and may provide adequate intakes assuming a composition of HM plus fortifier of 2.5 g protein/100 ml with a protein: energy ratio (PER) above 3.2 g/100 kcal [8]. There are a number of products available for fortifying HM for preterm babies which differ by the origin of milk used (bovine, human, or donkey), and by nutrient composition (multinutrient fortifiers or supplements of protein, lipids, carbohydrates). HM fortifiers have been produced to increase the nutritional content of HMH in order to meet the nutritional requirements of VLBW infants and to preserve the benefits HM [69, 70]. Through a process of lacto-engineering, HM can be fortified with skim and cream components derived from heat-treated, lyophilized mature DHM to produce a "human milk fortifier." This method of fortification avoids cow's milk proteins, but it is impractical, since it involves a complex process and requires a large supply of donor milk. When compared with premature infants fed preterm formula, infants fed exclusively fortified human milk had a significantly lower incidence of NEC and/or sepsis, had fewer positive blood cultures, and required less antibiotic administration [70–72]. Some for-profit companies have been set up to collect and buy HM, to manufacture, and to sell HM-based products. To treat huge volumes of HM (1200 L from 250 donors), they use Vat pasteurization (63 °C, ≥30 min). Vat differs from holder pasteurization, which is the commonly used method in non-profit HM banks. Vat pasteurization significantly reduced lactoferrin and total HM oligosaccharide concentrations when compared to Holder pasteurization. Human milk-based fortifier is obtained by concentrating heat-treated donor HM and then adding vitamins and minerals. Many studies suggested a benefit in terms of morbidity and mortality when babies are fed an exclusively human milk-based diet including HM-based fortifier, leading to a reduction of costs [68]. The OptiMoM study, recently published by O'Connor et al. [73], is the first trial comparing the efficacy of HM-based fortifier to bovine-based fortifier in the absence of formula. It is essential to evaluate the benefit-risk ratio, particularly as these products are very expensive and use large amounts of donated milk to make the fortifier which could be used more directly to feed preterm babies.

Preterm human milk, fortified with protein of bovine origin, has become the standard practice in most neonatal units. The composition of several fortifiers expressed per gram of protein varies both qualitatively and quantitatively [73]. In general, they contain bovine whey protein (intact or hydrolyzed), carbohydrates (mainly or exclusively glucose polymers or maltodextrins), minerals, and electrolytes such as sodium, calcium, phosphorus, and magnesium and some also contain micronutrients and vitamins.

The properties of human milk may be changed by nutrient fortification. Fortification may influence nutrient availability and may alter some biological properties of human milk. This is the result of the osmotic content of the fortifier but also of the rapid and continuous activity of human milk amylase on the dextrin content of fortifiers. High osmolality may induce abdominal discomfort and delayed gastric emptying. Recently, in order to avoid these problems, fortifiers with whole proteins, reduced carbohydrate content, and increased fat supplementation have been proposed [69, 70]. The presence of fat in fortifiers has the advantage of increasing the energy intake of human milk without increasing osmolality values.

The use of multinutrient fortifiers is associated with short-term improvements in weight gain and increments in both length and head circumference growth during hospital stay [70]. Although the fortification of human milk improves the general growth of preterm infants, growth of specific parameters such as lean body mass, fat mass, and bone mineral content is significantly lower in fortified human milk-fed infants than in those fed preterm formula [69, 70]. These differences could be related to the lower protein content of human milk compared with hypothetic values used to establish fortification. Indeed, the variability in expressed human milk with respect to its protein and energy content is high. This variability may result in undernutrition after standard fortification method. It has been demonstrated that individualized fortification may optimize protein and energy intake [69, 70]. Compared with standard fortification, individual fortification significantly reduces the variability in nutritional intakes, allowing the maintenance of protein intake and the PER in the range of the current recommendations. Further studies are advocated to verify the long-term effects of individualized fortification method.

In conclusion, early use of a human milk fortifier up to 1.3 g of protein per 100 ml may be recommended for more immature or smaller preterm infants, beginning from the time when they are able to tolerate 40–50 ml/kg/d of milk [8, 69].

Expressed Donor Human Milk

Expressed donor milk can be foremilk or hindmilk. These two types of milk respectively have lower or higher fat and

energy contents than milk received by the breastfed infant [68]. Mature donor milk will have a lower protein sodium, zinc, and copper content than that of milk produced in early lactation. Feeding donor breast milk is classically associated with poor weight gain and growth restriction. However, analysis of available evidences revealed that most of the studies considered were 20–30 years old and from an era when donor breast milk was fed without fortification or mineral supplements, often as the sole diet. It is not clear whether similar effects would be seen when supplementation with fortifiers is adopted. Additionally, infant fed donor breast milk had a significantly reduced risk of NEC [71, 74]. Treatment of donor breast milk (i.e., collection, store, froze, pasteurization, exposition to light) may induce qualitative alteration [74]. Breast milk in human milk banks is stored at low temperatures and subjected to thermal processing to guarantee its microbiological safety. Holder pasteurization (heating at 62.5 °C for 30 min and subsequent fast cooling) is the most frequent. Such pasteurization has been mainly assessed in terms of reduction in the activity of proteins of major biological relevance, such as immunoglobulins, lactoferrin, lysozyme, and gastrointestinal enzymes such as lipase. It decreases fat and energy content of human milk [74]. Frozen storage at −20 °C of pasteurized milk reduces fat, lactose, and energy content of human milk. These aspects may influence macronutrients and energy intake. Breast milk transmission of human immunodeficiency virus (HIV) is considered an important mode of neonatal infection [75]. Despite this fact, many researchers have observed that corresponding to the volume of milk consumed by the infant, maternal transmission via breast milk is still comparatively low. Some have noted the long latency period of breast milk HIV transmission with evidence of numerous anti-HIV factors in breast milk [75, 76]. The presence of HIV or of other viruses in maternal milk seem to be a requisite to spur immunological defenses to optimize necessary protection to the infant. Because the only intervention to prevent HIV transmission via human milk is not to breastfeed, the AAP recommends that HIV-infected mothers not breastfeed their infants regardless of maternal viral load and antiretroviral therapy. Although the use of human breast milk from HIV-infected mother is not recommended in industrialized countries, an HIV-infected woman receiving effective antiretroviral therapy with repeatedly undetectable HIV viral loads may choose to breastfeed in particular circumstances. This rare condition generally does not constitute grounds for an automatic referral to child protective services agencies. Infant HIV infection status should be monitored by nucleic acid amplification testing throughout lactation and at 1, 3, and 6 months after weaning. Some concerns about CMV infection in preterm infants receiving breast milk from seropositive mothers exist because CMV is excreted in breast milk in most lactating mother after a few weeks [72]. Differences in CMV acquisition from fresh or frozen milk have been suggested, and short-term high temperature pasteurization techniques were found to prevent CMV transmission more effectively than freezing [72]. Cryotreatment preserves the nutritional quality of human milk, but unfortunately not cancel the risk of CMV transmission. In contrast, compared with freezing, holder pasteurization and short-term heat inactivation for 5 s at 72 °C partially destroy the activity of some digestive enzymes, contemporarily eliminates viral infectivity in milk in each stage of lactation [74–76]. Decisions about breastfeeding of VLBW infants by mothers known to be CMV-seropositive should be made with consideration of the potential benefits of human milk versus the risk of CMV transmission.

Infant Formulas

Preterm Formula

When human milk is not available or extremely limited, cow's milk-based formulas for preterm infants must be used. Over the past 20 years, there has been a significant improvement in the nutrient composition of preterm formulas in order to meet the high nutritional needs of growing preterm infants. VLBW infants fed with these formulas showed an improvement of fat absorption, nitrogen retention, bone mineralization, and weight gain, when compared to VLBW infants fed standard term formulas or pooled human milk [8]. Although numerous consensus conferences have been organized, the optimal formula for VLBW infants has not yet been designed. European preterm formulas present several differences in the nutrient composition when compared to American formulas [8]. The latter have a higher MCT and a lower LCPUFA content, provide less lactose, and have a higher mineral intake. Nevertheless, according to more recent data, a general profile in macronutrients could probably be suggested. Energy content could be slightly higher than at present. Current recommendation for energy intakes is 110–135 kcal/kg/d for the ESPGHAN 2010 and 110–130 kcal/kg/d for experts' panel. According to the protein requirements, the protein content would represent 3.2–3.6 g/100 kcal, i.e., 2.4–3.2 g/100 ml [8, 15]. Whey-predominant protein with reduced glycomacropeptide and α-lactalbumin enrichment could be used to optimize the amino acid profile [30, 37]. Up to now, the use of protein-hydrolyzed formulas have not been recommended [38, 68]. The partition of the non-protein energy supply would favor the carbohydrate content up to 50–60% in view of its suggested benefits in sparing protein oxidation, enhancing growth and protein accretion, as well as improving quiet sleep and influencing the distribution of behavioral activity states in VLBW infants. The remaining energy supply would

be covered by fat with a fat blend carefully designed to reduce the long-chain saturated fatty acids content, and provide the essential fatty acids (LA and ALA) in an appropriate ratio as well as the LCPUFAs such as DHA and AA [8, 15]. MCT could be used with a maximum of 30–40% of lipids content [9]. Highly metabolizable (50–60% absorption rate) calcium content would be limited to 100–120 mg/100 ml. According to the expected nitrogen and calcium retention, the phosphorus content of this formula would represent 55–65 mg/100 ml, considering phosphorus absorption close to 90%.

Preterm formulas are available as powder or liquid in glass bottles or cans. Liquid formulas have the advantage that they provide sterile feeding thereby reducing possible infections reported in preterm infants fed powder formulas, such as by *Enterobacter sakazakii* [77]. Nevertheless, it is worth remembering that with liquid formulas, there is frequent discordance between claimed and available content of several nutrients. For instance, part of the calcium content of liquid formula may precipitate on the wall of the receptacle reducing the calcium actually available to the preterm infants. In addition, the heat treatment necessary to sterilize the liquid formulas reduces the bioavailability of various nutrients such as proteins, calcium, or copper. In consequence, when human milk is not available, powder formulas allowing adaptation of nutrient density and being of higher nutritional value remain the formulas of choice for the feeding of VLBW infants.

Hydrolyzed Formula

When human milk is not available for preterm infants, hydrolyzed formula is increasingly used as an alternative to standard cow's milk formula. Hydrolyzed formula is perceived as better tolerated and less likely to lead to serious complications such as necrotizing enterocolitis [78, 79]. However, hydrolyzed formulas are more expensive than standard formulas, and concern exists that their use in practice is not supported by high-quality evidence. Recent meta-analysis demonstrated that feeding hydrolyzed formulas to preterm infants was not associated with a lower risk of feeding intolerance defined as [20, 21, 28] a high mean prefeeding gastric residual volume necessitating enteral feeding discontinuation or [20, 21, 28] abdominal distention or other concerning gastrointestinal signs. There was no advantage of hydrolyzed formula use on NEC occurrence. Contemporarily, hydrolyzed formula was associated with slower weight gain. There were no associations of formula type with bone mineralization. No trials reported mortality rates, growth and neurodevelopmental outcomes, or risk of allergy or atopy beyond the initial hospital admission.

Post-Discharge Enteral Nutrition

Although there is no clear evidence on the added benefit of administering a nutrient-enriched diet to preterm infants after discharge, a few reviews suggest an improvement in growth parameters with no effect on neurodevelopmental outcomes [76]. Studies indicate that variations in dietary nutrient intake can contribute significantly to growth deficits in preterm infants, thus highlighting the choice of appropriate nutrition in this cohort [76]. In most neonatal units, the discharge of VLBW infants usually occurs when premature infants reach 35–36 weeks PMA and/or a weight of about 1800–2100 g. By that time, they have frequently accumulated energy, protein, and mineral deficits and develop a growth restriction. Additionally, they still present higher nutrient requirements than healthy term infants and premature infants born at the same PMA [78–81]. Protein- and mineral-enriched formulas have been proposed to feed preterm infants after discharge with the aim of minimizing as much as possible, postnatal growth restriction during the early weeks of life, inducing early catch-up growth, and reducing the adverse effects of early malnutrition [78–81]. Energy intakes are the main determinant of milk volume intakes. Compared to regular (standard) formula, a simple increase in energy density without changes in nutrients proportion had no influence on nutrients supply except in infants with limiting feeding self-sufficiency, not able to drink their quantity for several reason (bronchopulmonary dysplasia, cardiac insufficiency, neurologic insufficiency, "quickly tired" infants) [82]. In contrast, an increase in protein density with high PER influences protein intake and protein metabolism, and a positive effect of growth parameters was not observed in all studies [78–81]. The benefit of the nutrient-enriched formula on VLBW infants' growth was mainly seen during the early post-discharge period, between discharge and theoretical term (40 weeks PMA). This increased growth rate is observed in all preterm infants, even in those without postnatal growth deficit at the time of discharge. Additionally, there is a strong gender influence on the result of diet manipulation during the post-discharge period and the main positive results are more obvious in boys [68–70].

Considerable attention should also be focused on other specific nutrients, particularly calcium, phosphorus, iron, LCPUFAs, and vitamin A. Feeding post-discharge preterm infants formulas or HM with greater concentrations of calcium and phosphorus than those contained in term formula improves bone mineralization, particularly if the special formulas used during hospitalization are continued after hospital discharge [78–81]. However, the relative osteopenia of VLBW infants observed at the time of hospital discharge usually improves spontaneously in most VLBW infants after discharge, in a manner similar to that induced by the accel-

eration of growth at the first stage of adolescence. Therefore, provision of large amounts of calcium and phosphorous for long periods may not be necessary. With regard to vitamin D intake, there is no evidence that preterm infants should receive greater doses that term infants to maintain a normal plasma vitamin D concentration after discharge [82, 83]. Body iron stores are highly variable at discharge, so it is important to screen for iron deficiency at discharge and during the first year of life [63, 64]. Scientific societies recommend that preterm infants receive iron supplements for up to 1 year after discharge [63, 64]. Preterm infants fed HM should receive an iron supplement of 2 mg/kg/d by 1 month of age, and this should be continued at least until the infant is weaned to iron-fortified formula or begins eating complementary foods that supply 2 mg/kg/d of iron. Infants who have received iron loads from multiple transfusions of packed red blood cells may not require supplements. LCPUFAs need to be added in higher concentration in VLBW infants' feeds to improve brain development and the retina in particular [81–83]. Vitamin A status may be suboptimal in formula-fed VLBW infants for many months after discharge. Contrasting data exist regarding the utility of aggressive enteral supplementation with vitamin A (3000 IU per day) after discharge. At the moment, additional studies are needed to determine the dose and duration of vitamin A supplementation that allows infants to reach full repletion values [81–83].

Growth at 4 and 12 months and mineralization at 4 months after discharge are better in VLBW infants fed preterm formula during the first 2 months after discharge than in those fed term formula [81–83]. Protein and nutrient-enriched formula provided after term seems to improve the quality of growth in preterm infants. Infants fed nutrient-enriched formula have lower fat mass, corrected for body size at 6 months' corrected age, than infants fed standard formula or HM. Preterm infants fed nutrient-enriched formula after discharge exhibit an increase in lean body mass and peripheral fat mass but not central adiposity compared with infants fed term formula. These data indicate that nutrient-enriched formulas do not promote central adiposity in preterm infants, a feature that is associated with metabolic syndrome later in life.

On another side, high protein intake in early infancy in term infants could enhance the development of several diseases, such as obesity and hypertension later in life [2, 3]. Even if low protein intakes and poor growth during initial hospitalization in VLBW infants have been associated with poor adverse developmental outcomes, the safety of prolonged high protein intake in preterm infants after discharge has not been completely established. Thus, further studies are necessary to address this crucial safety aspect. Since early nutrition should not be considered simply in terms of weight gain but also for the biological effects with possibly lasting or lifelong significance, it seems prudent to suggest an enriched formula only in infants at risk of future growth failure.

Among the preterm infants, not all are at similar risk of later growth failure and adverse developmental outcomes after discharge. SGA infants and preterm infants that have developed a postnatal growth restriction seem to be the population at higher risk, boys in particular. In these infants, breastfeeding should always be promoted with or without a transient fortification of expressed HM up to 40 to 52 weeks PMA. If breastfeeding is not possible, a preterm formula or a special post-discharge formula that contains more protein, minerals, trace elements, and LCPUFAs should be preferred to a standard term formula until the preterm infant reaches between 40 and 52 weeks PMA to improve catch-up growth. AGA infants without growth restriction at the time of discharge from the hospital usually continue to maintain appropriate growth. In these infants, exclusive breastfeeding should be encouraged at discharge, and if not possible, a term infant formula with relatively low protein density (2.2 g/100 kcal) should be provided with particular attention to its LCPUFAs, mineral, and trace element content.

In conclusion, breastfeeding should always be encouraged in all preterm infants after discharge. In those with high risk of longitudinal growth restriction, fortified human milk or enriched formula (protein, energy, LCPUFA, micronutrients) may also be added to breastfeeding in order to improve the nutrient supply and to promote catch-up growth [79–83]. After discharge, growth should be monitored weekly to adapt feeding and to avoid growth failure, especially in VLBW infants.

Monitoring the Effects of Artificial Nutrition on Growth

Anthropometric Measurements

Monitoring nutritional status is required to detect nutritional deficits early and to guide nutritional supply in preterm infants under intensive care. Thus, nutritional assessment should be an essential skill of neonatal status caring for preterm infants [84]. Overall growth is monitored by anthropometry, as a proxy for body composition: measurement of body weight, length, head circumference, and, to a lesser extent, skin fold and arm circumference [84]. Anthropometric method to monitor growth and assess nutritional status in infants is rapid, inexpensive, suitable for bedside evaluation, and noninvasive despite limitations in the validity of several measurements in small infants [84].

Body weight, though largely used, does not inform on body compartment sizes. Weight gain or loss, reflects also

changes in body composition. In utero, the fetus experiences a decrease in extracellular fluid volume and an increase in lean tissue and fat mass as gestational age increases. The initial postnatal weight loss is attributed to contraction of body water compartments and catabolism of endogenous glycogen, fat stores, and lean tissue in the absence of adequate energy and nutrients supply. Physiologic postnatal weight loss is 5–10% of total body weight. Maximum initial weight loss usually reaches its nadir by 3–6 days of life and birth weight is usually regained by 7–14 days of age. Body weight should be measured daily to assess growth and fluid and electrolytes status in order to define optimal management. Once birth weight is regained, subsequent weight gain of 17–20 g/kg/d is desirable for infants up to 32 weeks PMA. Weight gain should be evaluated weekly to identify infants with average weight gains of <15 or >25 g/kg/d. Unfortunately, poor weight gain is common in infants with extreme prematurity or pathological conditions (chronic lung disease, severe intraventricular hemorrhage, necrotizing enterocolitis, and late-onset sepsis). Postnatal growth restriction is mainly due to insufficient nutritional support and can be avoided in most VLBW infants receiving adequate nutritional intakes [84, 85].

Anthropometric measurements should be plotted on percentile growth curves for comparison with established reference data. Plotting weight on classic intrauterine growth charts can determine whether an infant is small for gestational age (SGA, weight <10th percentile or <2 standard deviation score), appropriate for gestational age (AGA), or large for gestational age (LGA weight >90th percentile or >2 standard deviation score). Growth restriction is usually defined for infants who cross (decrease) the percentile curves, when their standard deviation score decrease of more than 0.6 or 1.0, or when AGA body weight become SGA. Using the growth chart, optimal weight for length is identified by finding the weight, which is approximately on the same percentile as the infant's length measurement. Current weight expressed as a percentage of optimal weight for length can be used to identify infants at risk for under- or overnutrition [85]. Term neonates use to accumulate significant fat mass during the first months of life, and it is not known whether preterm neonates need to accumulate the same amount of fat mass during their first months of life, which would be significantly different than fetuses of the same PMA. Although most experts agree with the statement that low weight-for-length status correspond to growth restriction, it is not known what is the optimal weight-for-length status for preterm infant when they are plotted on intrauterine growth charts.

Neonates with a history of intrauterine growth restriction may have growth delays in weight, length, or head circumference. When intrauterine growth retardation (IUGR) occurs early in pregnancy, frequently symmetric or proportionate growth restriction is observed. In this case, all growth parameters are affected, resulting in a greater risk of neonatal morbidity, future growth problems, and neurodevelopmental delay. In the late IUGR, more often only one parameter displays impaired growth. If weight is low compared with length and head circumference, catch-up growth may be expected. When head circumference is low compared with weight and length, neurological or developmental impairment may occur.

Fenton's charts were designed to commence monitoring infants at 22 weeks' PMA and continue for 10 weeks post term according to the gender [85, 86]. Nevertheless, charts that do not take into account the initial physiological weight loss, such as the Fenton 2013 charts, are not appropriate to monitor short-term postnatal growth [84]. A large international longitudinal observational study showed that preterm infants with uncomplicated postnatal adaptation have transitioned to a weight gain trajectory of 0.8 standard deviations below birth weight at the 21st postnatal day [84]. Based on these data, it seems more useful for monitoring intrahospital growth a free-access online growth calculator (https://www.growthcalculator.org/) that graphically shows current percentiles, target weight, and deviation of the current weight in grams.

It is not easy to assess lean body mass in preterm newborns, especially in the presence of edema or dehydration. Many tools have been tested to better define body composition. Regional anthropometry, triceps skinfold, and mid-upper arm circumference which estimates body adiposity have been proposed as predictors of infant body composition. Standards are available for infants between 24 and 41 weeks of gestation, and they can be used to compare those of an individual infant with reference values or to assess individual changes over time [87]. However, intra- and inter-examiner measurement technique variability and positioning of infants can contribute to measurement of errors. In addition, the use of calipers to measure triceps skinfold may not be feasible in extremely immature infants who have delicate, easily punctured skin. For these reasons, regional anthropometry is not routinely assessed in preterm infants.

Laboratory and Biomedical Tools

Serum nutrient levels can be used as markers of nutritional status in VLBW infants, but many factors not related to nutrition can alter laboratory results and must be considered when interpreting biological data. Technical factors, such as storage and processing of the specimen, type of laboratory method used, and technician accuracy, may affect the validity of laboratory tests. Blood urea nitrogen (BUN), a by-product of protein degradation, is frequently used to monitor protein intake in premature infant feds with human milk,

after the first month of life [84]. However, BUN does not measure protein nutritional status but reflects dietary intakes, hydration status, renal function, and the presence of catabolism. Serum concentrations of transport proteins albumin, transferrin, prealbumin (transthyretin), and retinol-binding protein (RBP) have also been proposed as indicators of protein nutritional status [84]. Both albumin and transferrin have a longer half-life than prealbumin or RBP; thus, serum concentrations of different transport proteins reflect different nutritional intake at different time periods. Prealbumin and RBP concentrations appear to correlate better with nitrogen balance during artificial nutritional therapy. Serum transferrin and RBP levels may be influenced by suboptimal iron, zinc, or vitamin A status. Low values of these proteins are usually described in premature infants during the first 3 months of life when compared to term infants suggesting insufficient nutritional support in premature infants. However, if transport protein concentrations are normal but growth is poor, nutritional supply need to be increased to meet the nutritional demands of VLBW infants.

Alkaline phosphatase (ALP) is an enzyme predominantly produced in liver and bone. It may be elevated during normal growth conditions, liver diseases, or bone diseases. In preterm neonates, an increase of ALP may suggest the presence of an osteopenia of prematurity secondary to calcium, phosphorous, magnesium, or vitamin D deficiencies [84]. As previously discussed, hypophosphatemia and hypercalciuria should also be regularly ruled out to avoid severe osteopenia.

Iron deficiency is frequent in premature infants and typically occurs after 4–8 weeks. Serum ferritin may be used as a good marker of iron store and values above 116 μg/l are advised. Indeed, iron deficiency anemia and low ferritin stores below 75 μg/l have been associated with nonoptimal developmental outcomes. Classical prevention strategy of iron deficiency in preterm infants are delayed (30–120 s) cord clamping at birth and systematic 2–3 mg/kg/d supplementation from the third week of life. On another side, excessive iron intakes may be toxic leading to some oxidative stress, increased risk of infection and retinopathy, and poor growth without any advantages on developmental outcomes. High ferritin concentration above 400 μg/l might be considered as iron overload and iron intakes should be decreased [84].

Despite premature infants are at high risk for developing complications from deficiencies of zinc, vitamin B12, folate, vitamin E, and copper, laboratory tests used for diagnosis of vitamin deficiency are expensive and should be reserved to the infants at high risk of deficiencies: malabsorptive diseases and long-term (>3 weeks) parenteral nutrition [84].

Tracking body composition rather than just body weight is more accurate for monitoring nutritional status and improving nutritional outcomes [84, 88]. Dual-energy X-ray absorptiometry (DXA) and air displacement plethysmography (ADP) are validated and convenient methods for body composition assessment in small infants, since they are noninvasive, rapid to perform, and not affected by movements. However, these methods are expensive and not accessible for the majority of clinical settings.

Advances in imaging techniques have allowed more direct in vivo measurement of body composition. Dual-energy x-ray absorptiometry (DEXA), requiring minimum radiation exposure (<0.3 mrem), has been successfully used for measurement of lean mass, fat mass, and bone mineral content in newborns. DEXA remains the most widely used method for the in vivo measurement of whole-body composition in humans. However, there are concerns on the accuracy in VLBW infants, especially in regard to determination of fat mass. In particular, the diagnostic power of this method for body composition determination depends on model of device, software, and scanning mode. These aspects limit the possibility to use DEXA reference ranges for preterm neonates. Nowadays, air displacement plethysmography has recently emerged as a noninvasive technique to determine body fat mass and fat free mass in preterm infants [84, 88]. It is based on the measurement of body volume using gas laws, and several studies have confirmed the reliability and accuracy of air displacement plethysmography in animals, infants, and neonates [84, 88]. Further validation of these techniques is advocated for routine use.

Conclusions

Enteral nutrition is crucial to meet nutritional requirements of preterm neonates. HM is considered the first choice in preterm infants feeding. Each NICU should make strong efforts to promote lactation. Nutritional support relies on both PN and EN, bearing in mind that the parenteral route functions as a support not necessitating the cover of full requirements in the early adaptive period.

Concerns regarding FI and the perceived risk of NEC are the main obstacles for initiation and advancement of enteral feeds in VLBW infants in NICU. The appropriate management of feeding intolerance is advocated in order to reduce the risk of complications, such as NEC, a delay establishment of full enteral feeding, and the length of hospitalization.

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Parenteral Nutrition in Premature Infants

7

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Introduction

This chapter on parenteral nutrition (PN) in premature infants is an update of the previous chapter [1] based on current body of evidence. The work of the authors of the first version is gratefully acknowledged.

Very premature infants (<32 weeks gestational age) have an immature gastrointestinal tract, and the combined use of enteral and parenteral nutrition (PN) is frequently required to cover nutrient needs during the early days or weeks of life. Studies on PN practices show that nutritional management varies widely and is often nonoptimal [2–8]. The impact of perinatal undernutrition on growth and brain development has been well known for years from both human and animal studies [9–12]. In premature infants, observational cohort studies and randomized controlled trials suggest that postnatal protein and energy malnutrition increase the severity of postnatal diseases and induce postnatal growth restriction, inadequate brain development, and poor neurodevelopmental outcomes [10, 13–24]. Several of these studies have evaluated how to optimize PN support in premature infants and thereby improve early energy and protein supplies. They confirm that preterm infants have a high risk of cumulative energy and protein deficits during the first days of life [10], but also that the parenteral weaning phase requires special attention [6, 7]. Optimal parenteral nutrient intakes may further be compromised by degradation/oxidation of the intravenous lipid emulsion (IVLE) or the fat-soluble vitamins given, destabilization of the emulsions used, precipitations, or by metabolic disturbances including electrolyte imbalances, osteopenia of prematurity, and hyperglycemia [25]. Other challenges of PN are prescription, transcription, or validation errors, the mix up of single products, errors at the infusion site, catheter misplacement or extravasation of the PN solution, and catheter-related septicemia [26–30].

To help harmonize nutritional practices and optimize clinical outcomes, both in the short and the long run, international guidelines on the provision of PN in children, including preterm infants, have been published [31] and revised [32]. The guidelines cover macro- and micronutrient requirements, common complications, and important organizational points [28–30, 33–43]. This chapter discusses the most important features regarding PN in premature infants, summarizing practical aspects and best practice recommendations.

The Standard for Premature Infants Growth

Growth Rates

Fetal growth rate is extremely high during the third trimester of gestation and much greater than during any other periods of life (Fig. 7.1). If the mean fetal weight gain during the last trimester of gestation is 15–17 g/kg/day, it must be emphasized that fetal weight gain usually decreases from ~20 g/kg/day at 24–28 weeks of gestation to ~10 g/kg/day at 39–40 weeks [44]. The optimal growth pattern for premature infants has not been defined, but generally a target growth similar to that of the fetus of similar gestational age (GA) up to term equivalent age (TEA) is recommended [45, 46], followed by growth similar to that of the breast-fed term infant afterwards [47]. However, fetal growth charts do not incorporate the physiological postnatal weight loss and are thus not optimal for

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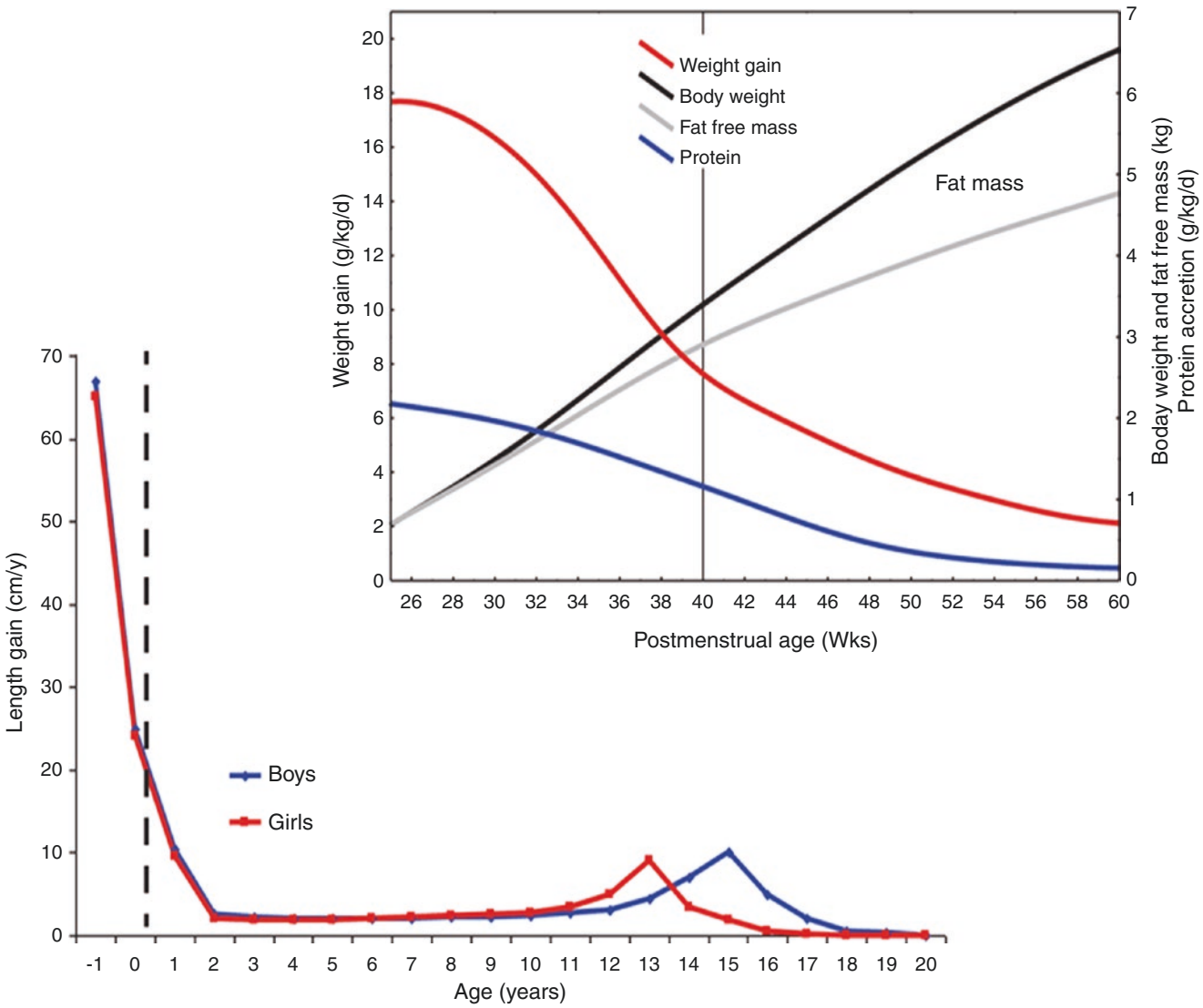

Fig. 7.1 Perinatal growth and changes in weight gain, body composition, and protein accretion

monitoring growth [44, 48]. Since healthy preterm infants usually adapt to their new trajectories (on average 0.8 SD below their birth weight percentile) within the first 3 weeks of life, Rochow et al. created individual based postnatal growth trajectories [49, 50] (Fig. 7.2). These are based on the respective day-specific median growth velocities obtained from day 21 of life from the Fenton growth chart adjusted with a correction factor so that the preterm and the term growth trajectories gradually merge around TEA (42 + 0/7 days). This concept may be a promising approach to monitor growth and adapt nutritional care, but needs validation [50]. At present the growth trajectory calculator is available online at http://www.growthcalculator.org/ (Rochow N et al., Rochow N, Landau-Crangle E, Thommandram A, Fusch C. Individualized postnatal growth trajectory for preterm infants – online calculator. 2016; Accessed August 2020).

Body Weight Composition

The body weight composition changes during the last trimester of pregnancy. Both intrauterine and extrauterine growth is physiologically associated with an increase in fat-mass deposition (Fig. 7.1). Advanced methods for body composition assessments such as dual energy X-ray absorptiometry (DXA) and air displacement plethysmography (ADP) are not available in most neonatal intensive care units. However, if performed with appropriate techniques and instrumentation, direct anthropometric measurement such as body weight, length, head circumference (HC), mid-upper arm circumference (MUAC), and skinfolds may be used with derived measurements (e.g., MUAC:HC, weight to length ratio) to estimate body composition [51]: skinfolds reasonably estimate body fat, whereas body length reflects skeletal growth and fat-free mass.

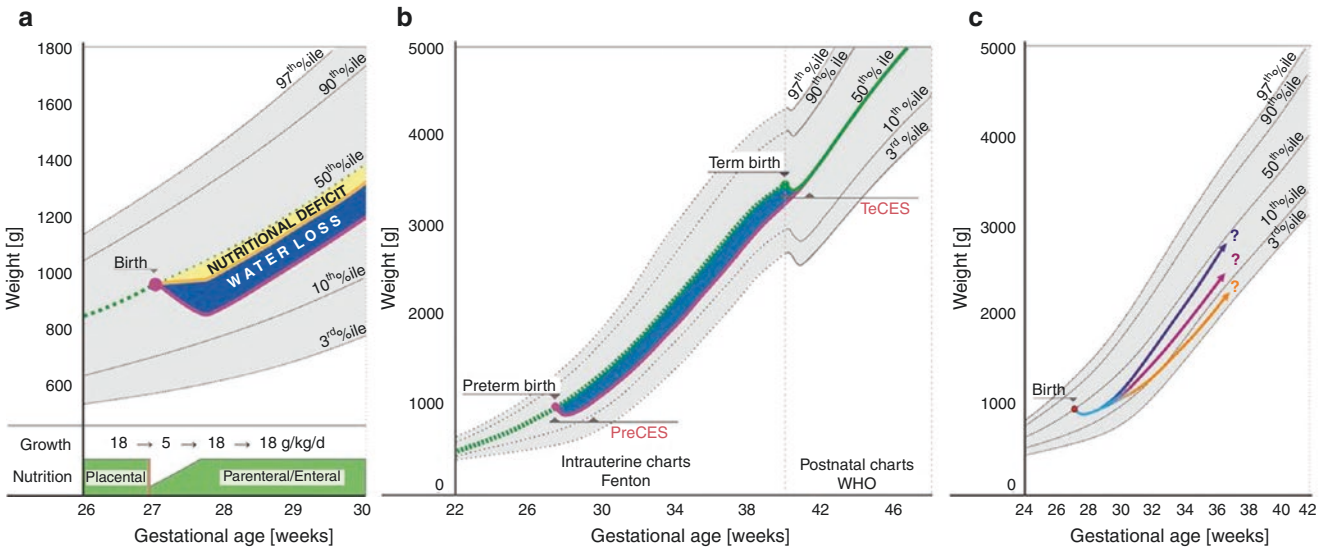

Fig. 7.2 Reproduced from Ref. [50] Use of common growth charts. (**a**) Physiologic weight loss during postnatal adaptation. Minor contribution from temporarily decreased nutrient intake (yellow area), major contribution from contraction of extracellular space with water loss (blue). (**b**) Postnatal offset of growth trajectories of preterm infants and convergence of preterm with term trajectories at 42 + 0/7 weeks; dotted curves of intrauterine trajectories obtained from a meta-analysis of birth weight percentiles, and solid-line postnatal trajectories represent preterm (purple) merging with term (green). One-time postnatal contraction of extracellular water spaces can either occur at term (TeCES) or after preterm birth (PreCES), leading to a temporary separation of growth curves by the equivalent of approximately −0.8 z-scores and remerging after 42 + 0/7 weeks. (**c**) Current growth charts with lack of target trajectories

Nutritional Support in Premature Infants

Nutrition of preterm infants may be divided into two distinct periods: the immediate adaptive or "transitional" period during the first 3–7 days of life and a stable "growing" period up to discharge from the neonatal intensive care unit (NICU). The transitional period, including the immediate postnatal adaptation of the premature newborn to the extrauterine environment, may be prolonged in very immature infants or in infants with major clinical disorders. Immature organs and accompanying morbidities influence nutrient intakes as well as the metabolism of the nutrients provided [2, 3, 8, 52]. During the adaptive and transitional period, most of these infants require PN support, but recent studies show that very and extremely preterm infants may reach full enteral feeds by 7–14 days [21, 53, 54]. Moreover, these studies highlight the importance of reducing the transitional period by more rapidly providing sufficient intakes to promote anabolism and to reach stable-growing requirements [6, 21, 54–56].

Energy

Postnatal Energy Metabolism

The Atwater's factors are usually used to calculate the metabolizable energy contents and intakes both in PN and enteral nutrition. However, the gross energy available after complete combustion of the respective macronutrients varies somewhat. For instance, the gross energy content of 1 g of amino acid (AA, ~4.75 kcal/g) is about 10% lower than that of 1 g of protein (~5.25 kcal/g), the gross and metabolizable energy content of glucose (~3.75 kcal/g) is less than that of more complex carbohydrates (~4 kcal/g), whereas for intravenous lipid emulsions (IVLE), metabolizable energy content is similar to gross energy (~10 kcal/g including glycerol energy content) but could be lower in IVLE containing medium-chain triglycerides (MCT) [1, 57, 58]. These differences are not easy to incorporate into practice.

The energy requirements for premature infants correspond to the sum of energy needed to support all basal functions, including thermoregulation and resting muscular activity (resting energy expenditure, REE), plus the energy needed for growth. The proportion of energy needed for physical activity is often negligible in extremely preterm infants because they usually sleep or lay comfortably wrapped. Energy expenditure measured by indirect calorimetry increases slightly with postnatal age and varies from 45 to 55 kcal/kg/day. The energy cost of growth includes the cost of tissue synthesis and the energy stored in the new accreted tissues such as fat mass and lean body mass (LBM). Estimating a postnatal weight gain of 17–20 g/kg/day accompanied by adequate LBM accretion, the extra energy needed for growth would be 50–70 kcal/kg/day in premature infants. Therefore, metabolizable energy requirements for premature infants on PN are calcu-

lated to be between 90 and 120 kcal/kg/day [33]. Taking into account the commonly encountered cumulative energy deficits and a potential need for catch-up growth, energy intakes higher than the upper recommendation may be needed.

Recommendations for Energy Supply During Total PN

Current PN recommendations for preterm infants suggest providing a minimum of 45–55 kcal/kg/day on the first day of life followed by a gradual increase to 90–120 kcal/kg/day within the first week of life [33]. The energy intakes need to be adjusted according to growth and metabolism during the stable growing period (Table 7.1).

Recommendations for Energy Supply During Partial PN

Nutritional recommendations promote early initiation of enteral feeding in premature infants [59]. However, since absorption may vary and feeding volumes below 25 mL/kg/day mainly serve as trophic gut feeding, these volumes are usually not included in the calculation of total energy intakes. Thereafter, when feeding increases total energy intakes need to consider the sum of both parenteral and enteral intakes. Due to lower absorption rates with enteral nutrition (80–90%) [58], recommended enteral energy intakes are generally 10–20% higher [45].

Table 7.1 Recommended nutrient intakes for premature infants requiring parenteral nutrition

	Early phase	Transition	Growing phase
Water (mL/kg/day)	60–100	120–180	140–160
Energy (kcal/kg/day)	45–55	75–100	90–120
Amino acids (g/kg/day)	1.5–2.5	2.5–3.5	2.5–3.5
Glucose (g/kg/day)	6–8	6–10	11.5–14.5 (17)
Lipids (g/kg/day)	1–2	2–3	3–4
Sodium (mmol/kg/day)	0–2 (3)	2–5 (7)	3–5 (7)
Potassium (mmol/kg/day)	(0)1–3	1–3	1–3 (5)
Chloride (mmol/kg/day)	0–3	2–5	3–5
Calcium (mmol/kg/day)	0.8–2.0	1.5–2.5	1.6–3.5
Phosphorus (mmol/kg/day)	1.0–2.0	1.5–2.5	1.6–3.5
Magnesium (mmol/kg/day)	0.1–0.2	0.1–0.3	0.2–0. 3

Adapted from the ESPGHAN 2018 guidelines. PN should always be accompanied by provision of vitamins and trace elements

Amino Acids

Intravenous AA Solutions

Considerable improvements in intravenous AA solutions have been achieved since the 1960s. Specific pediatric AA solutions were designed in the early 1990s with high essential and conditionally essential AA content for use in premature infants [60, 61]. Three different standards of AA profile have been suggested for premature infants: umbilical fetal cord blood AA during last trimester of gestation, healthy breast-fed term infant's plasma AA, and human milk AA composition [34]. Due to the poor solubility of tyrosine and cystine, current AA solutions have some relative AA imbalance compared to enteral nutrition [61]. The ideal intravenous AA mixture for PN in premature infants is still a matter of debate. Nevertheless, biochemical tolerance and nitrogen utilization in infants do not change significantly despite the different compositions of current pediatric intravenous AA solutions [1, 60]. There is also no evidence to show that one solution has better growth or long-term outcomes as compared to another [61].

Postnatal AA Requirements

Fetal protein accretion is estimated around 2–2.5 g/kg/day during the last trimester of gestation [62]. Isotope studies in animals and in human fetuses have demonstrated that fetal AAs are not exclusively used for protein synthesis but also serve as an energy source by oxidation [63]. The fetal AA uptake during the last trimester of gestation has been estimated to be between 3.5 and 4.5 g/kg/day up to term [62]. Similarly, postnatal nitrogen balance studies in premature infants on PN have shown that an AA intake of 1.5–2.0 g/kg/day from the first day of life is needed to avoid a negative nitrogen balance [64]. Based on fetal accretion rates, AA intakes between 3.5 and 4.0 g/kg/day have been advocated during the stable growing period [31, 60] to promote growth and long-term neurological outcomes. However, aside from less postnatal growth failure [61, 65] and improved nitrogen/protein balance [61, 65, 66], recent systematic reviews and meta-analyses do not find clear beneficial effects with early higher or high dose AA intakes (>3.5 g/kg/d) [61, 65, 66]. In fact, studies on the safety of high AA intakes, with or without a balanced energy intake, are conflicting, but early and higher AA intakes increases the risk of abnormal blood urea nitrogen (BUN) concentrations and metabolic acidosis [65, 67, 68].

Even though BUN is frequently used to evaluate the adequacy of protein intakes in preterm infants, considering that BUN reflects protein degradation and AA oxidation, increased BUN concentrations may be due to several other reasons such as acute kidney injury, dehydration, inadequate concomitant energy intakes, or suboptimal amino acid composition. Increased BUN concentrations may also reflect higher protein intakes resulting in enhanced amino acid oxidation [61]. Therefore, attention is needed, when BUN levels are used as a marker of protein or AA overload during the first week of life in premature infants.

Recommendations for AA Supply

Practical recommendations for premature infants on PN are to provide (1.5–2.5) g/kg/day of AA on the first day of life and to gradually increase AA intake up to 2.5–3.5 g/kg/day [34]. AA intakes ≥2.5 g/kg/d should be accompanied by nonprotein energy intakes >65 kcal/kg/d and adequate micronutrient intakes to avoid hypophosphatemia and neonatal refeeding syndrome [69–71] (Table 7.1).

Carbohydrates

Intravenous Carbohydrates Solutions

Glucose is the only intravenous carbohydrate used for nutritional support with the exception of the glycerol content in IVLE. Early provision of carbohydrate supply is required to prevent hypoglycemia in premature infants. Glucose is readily available for brain metabolism and represents its main source of energy during PN.

Postnatal Glucose Metabolism

Early postnatal glucose infusion is essential in very preterm infants and an intake of 6–7 g/kg/day (4.2–4.9 mg/kg/min) is necessary to prevent early postnatal hypoglycemia resulting from the interruption of the materno-foetal glucose transfer and the low glycogen reserves of premature infants [36, 72].

After birth, glucose metabolism is frequently impaired as the mechanisms for glucose homeostasis are still immature. The endogenous glucose production is not completely suppressed by glucose intakes and the maximal glucose oxidation rate is generally limited to 17 g/kg/day (12 mg/kg/min) in term infants, but may be less in very preterm infants; <12 g/kg/d (8 mg/kg/min) [36, 72]. Very preterm infants are thus not only at risk of early hypoglycemia but also prone to hyperglycemia, in particular during PN. The incidence of hyperglycemia increases with decreasing gestational age and has been associated with increased mortality and neonatal morbidities like nosocomial infections, intraventricular hemorrhage, necrotizing enterocolitis, and impaired neurodevelopment [73, 74]. However, causality has not been proven in RCTs. When glucose intake exceeds total energy expenditure and the capacity for oxidation (energy production), excessive glucose is converted to fat (*de novolipogenesis*) [75]. This is an energy-consuming process which may increase CO_2 production and minute ventilation [76–78].

Hypo- and Hyperglycemia in Premature Infants

The definition of hypo- and hyperglycemia, as well as long-term consequences of these impairments, remains controversial. Reference plasma glucose concentrations are generally defined between 2.6 mmol/L (0.47 g/L) and 6.6 mmol/L (1.2 g/L). Hypoglycemia is regarded a metabolic emergency that needs to be rapidly corrected, most commonly by slow infusion of a glucose bolus (200 mg/kg = 2 mL/kg of 10% glucose solution) and by increasing nutritional intakes.

Premature infants on PN receive glucose at a continuous infusion rate and may thus often have plasma glucose concentrations higher than 6.6 mmol/L. A plasma glucose concentration up to 10 mmol/L (180 mg/dL) is usually well tolerated, but as mentioned earlier, higher concentrations are associated with increased mortality and morbidity [73, 74]. Moreover, hyperglycemia-induced glucosuria may cause osmotic diuresis, dehydration, and plasma hyperosmolarity [79, 80]. When faced with hyperglycemia, various contributing factors should be evaluated and properly addressed (i.e., high glucose and energy intakes, hypophosphatemia, stress, sepsis, pain, dehydration, ongoing intraventricular hemorrhage, and steroid treatment). Increasing AA intakes or the protein to energy ratio may contribute to improved glucose tolerance [65], but often total glucose intake needs to be reduced (10–15%) for a transient period of time. If blood glucose levels persist >10 mmol/L despite reasonable adaptation of the glucose infusion rate, insulin therapy is recommended [36]. Insulin may be initiated at a dosage between 0.02 and 0.05 IU/kg/h, but careful monitoring and appropriate adjustments are needed to avoid hypoglycemia [1, 80]. If not critically ill, adequate protein and energy intakes should be maintained to avoid nutritional deficits resulting in postnatal growth restriction.

Recommendations for Glucose Supply

It is recommended to start intravenous infusion of glucose at 4–5.5 mg/kg/min (6–8 g/kg/d) as soon as possible after birth to avoid hypoglycemia in very preterm infants or in infants at high risk of hypoglycemia (severe growth restriction, maternal diabetes) [36]. During the transitional period, intakes may gradually be increased up to 11.5–14.4 g/kg/day (8.0–10.0 mg/kg/min) according to tolerance, in order to provide adequate energy intakes (Table 7.1). The maximum glucose intake should not exceed the maximum glucose oxidation rate (12 mg/kg/min) or more than 60–75% of nonprotein energy intakes.

Lipids

Intravenous Lipid Emulsions

Intravenous lipid emulsions (IVLE) are important constituents of PN because lipids are high-density energy substrates, important building blocks for cellular components, and provide the essential polyunsaturated fatty acids (PUFAs), linoleic acid (omega-6), and α-linolenic acid (omega-3) [81, 82]. Linoleic acid is converted to arachidonic acid (ARA) and linoleic acid to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). ARA and DHA account for ~25% of the total fatty acids of the brain, predominantly as structural components of neural cellular membranes [83]. Since preterm infants have low endogenous capacity for conversion of linoleic acid to ARA and alpha-linolenic acid to DHA and EPA [84], and these fatty acids accumulate rapidly during the last trimester and in early life, that is, the period of brain growth spurt [81, 84, 85], ARA and DHA are considered conditional essential for preterm infants. AA, DHA, and EPA are also precursors of prostaglandins and bioactive eicosanoids (lipoxins, resolvins, protectins, and maresins) that hold pro- and anti-inflammatory properties [82, 86].

Current commercially available IVLE are shown in Table 7.2. These IVLE differ by the source of their fatty acids (soy, safflower, coconut, olive, and/or fish oil) and their composition, tocopherol (vitamin E) and phytosterol content (Table 7.2) [87]. The conventional soybean oil-based IVLE has a high content of phytosterols and PUFAs (predominantly linoleic acid), which are considered to contribute to intestinal failure/PN associated liver disease (IF/PNALD) and to sustain inflammation.

The fatty acid composition of the newer IVLE have been developed to improve lipid clearance, reduce lipid peroxidation, and provide fatty acids that are either more immunological inert or evoke anti-inflammatory mechanisms [86]. Since very preterm infants are at risk of inflammation related neonatal comorbidities, there has been strong expectations for beneficial effects with newer IVLE. However, studies with composite IVLE are inconsistent in regard to their effects in reducing major preterm morbidities. The most

Table 7.2 Commercially manufactured intravenous lipid emulsions

Abbreviation	Intralipid 20% SO	ClinOleic 20% OO/SO	Lipofundin 20% MCT/SO	SMOFlipid 20% multicomponent FO-containing	Omegaven 10% FO
Year of introduction	1960s	1990s	1980s	2000s	1990s
	Oil source (%)				
Sovit bean	100	20	50	30	0
MCT	0	0	50	30	0
Olive	0	80	0	25	0
Fish	0	0	0	15	100
	Fatty acids (% of total fatty acid)				
Linoleic acid	53	18.7	29.1	37.2	4.4
Arachidonic acid	0.1	0.5	0.2	1.0	2.1
α-Linolenic acid	8	2.3	4.5	4.7	1.8
Eicosapentaenoic acid	0	0	0	4.7	19.2
Docosahexaenoic acid	0	0	0	4.4	12.1
n-6:n-3 ratio	7:1	9:1	7:1	2.5:1	1:8
Phytosterols (mg/L) based on Angsten et al [39][a]	348 ± 33	237 ± 8	NA	47.6	0
Phytosterols (mg/L) based on Xu et al [27][b]	439.07 ± 5.72	274.38 ± 2.60	278.14 ± 5.09	207	0
α-Tocopherol (mg/L)	38	32	85 ± 20	200	150–296

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FO fish oil, *MCT* medium-chain triglycerides, *OO* olive oil, *SO* soya bean oil

[a]Data in the table are the mean value when an interval is given from the manufacturer

[b]Independently evaluated concentration of nine different phytosterols and squalene

recent updated Cochrane review on IVLE for preterm infants, which included 29 studies and 2037 infants, did not find that any IVLE was more beneficial than any other for prevention of PNALD/cholestasis, growth, mortality, or any other neonatal outcome [88]. In patients with surgical conditions or established cholestasis, there was insufficient evidence to determine with any certainty whether fish-oil containing LEs offer any advantage in prevention or resolution of cholestasis or other clinical outcomes. Further research is needed to determine the advantages of IVLE with fish oil compared to other mixed IVLE, and to determine the safety of providing an unbalanced ratio of DHA (and EPA) to ARA in premature infants [89–92]. Meanwhile, due to the less balanced nutrition provided by pure soybean oil IVLE, it is recommended to use composite IVLEs with or without fish oil for PN lasting longer than a few days [35]. In cases of intestinal failure or PN associated liver disease, a reduction of IVLE dosage and/or the use of a fish-oil containing IVLE should be considered along with other measures to reduce common risk factors of liver disease.

Postnatal Lipid Metabolism

Lipid oxidation depends on lipid intakes, energy intakes, and energy needs for metabolism. During PN, lipid oxidation is inversely related to glucose intakes [75, 93]. If glucose is provided at a rate higher than the maximum glucose oxidation rate, oxidation of fatty acids ceases and glucose is converted to fat. Respiratory load and carbon dioxide production may be lowered when a part of energy intakes is provided by IVLE instead of a high proportion of glucose. Maximum lipid oxidation in neonates usually occurs when IVLE intakes provide 40% of nonprotein energy intakes, corresponding to 1 g of lipid for 3.6 g of glucose. Additionally, it has been suggested that nitrogen retention could be improved by adding IVLE to PN [31].

IVLE can be used from the first days of life and should be provided as an integral part of PN [35, 94, 95]. Since IVLE are isotonic they also reduce the osmolarity of the PN solution, making them more suitable for infusion in peripheral veins [31]. Continuous lipid infusion is generally preferred in neonates, but assessment of plasma triglyceride levels need to be monitored to avoid hyperlipidemia, particularly in small for gestational age (SGA) infants, and in neonates with high lipid intakes, hyperglycemia, sepsis, hypoxemia, or severe hyperbilirubinemia. Even if there are some controversies about the level of maximal plasma triglycerides tolerance in premature infants, there is general consensus that lipid intakes should be reduced when plasma triglycerides concentrations exceed 3.0 mmol/L (265 mg/dL) during continuous IVLE infusion [35]. However, since excess glucose intake may cause hypertriglyceridemia, glucose load should be considered before adjustments in lipid intakes are undertaken.

The use of carnitine supplementation during PN in premature infants is controversial. Carnitine is necessary for the transport of long-chain fatty acids across the mitochondrial membranes before fatty acids are utilized by beta-oxidization [35]. Carnitine is not routinely included in commercial PN solutions, and it has been demonstrated that in parenterally fed infants, plasma and tissue carnitine levels decline with postnatal age, suggestive of insufficient carnitine intake [96]. However, beneficial effects of parenteral carnitine supplementation on clinical outcomes are inconsistent and current recommendation is to consider carnitine supplementation on an individual basis (10–20 mg/kg/d) [35].

Recommendations for Lipid Supply

Lipid intakes during PN should represent 25–40% of nonprotein energy intakes in order to promote lipid oxidation and to reduce lipid deposition in fat mass. Current recommendations encourage the provision of IVLE as soon as possible after birth in all premature infants at a dosage of 1–2 g/kg/day. This dosage ensures sufficient provision of the essential fatty acids linoleic and α-linolenic acid. During the transitional postnatal period, IVLE can further be increased by 0.5–1 g/kg/day up to 3–4 g/kg/day according to metabolic tolerance (Table 7.1).

Fluids and Electrolytes

Postnatal Fluid and Electrolytes Metabolism

Birth is associated with major changes in water and sodium homeostasis. During the early transition phase, a physiologic contraction of extracellular fluid (ECF) volume takes place, and a net water and sodium loss is wanted – resulting in the "postnatal physiological weight loss of the newborn" [37, 97]. Due to renal immaturity, fluid and electrolyte disturbances are frequently observed in very preterm infants during this period.

Several mechanisms are responsible for the impaired water and sodium homeostasis in very preterm infants, including reduced glomerular filtration rate (GFR) and immature tubules [37, 98–100]. Compared to term infants, premature infants have higher transepidermal and insensible water losses. When these water losses are not anticipated or inadequately corrected for, the infants are put at risk of volume depletion and hypernatremia because the immature kidney has limited urine concentration ability [98–100]. In addition to reduced urine concentration ability, the preterm newborn infant also has limited ability to excrete an enhanced

water and sodium load. Following birth, developmental changes in glomerular function and tubular sodium handling occur that are mostly dependent upon gestational age. Indeed, postnatal maturation of GFR is less marked in the most premature infants compared with term infants, and GFR can be impaired by several factors and clinical conditions during the adaptation to extrauterine life [100].

High and low, but also large fluctuations in sodium concentrations have been associated with increased morbidity and mortality, including compromised cardio-respiratory functions and adverse developmental outcomes in preterm infants [101–106]. Altered sodium concentrations may be due to dehydration (weight loss above 10%) combined with or without inadvertent high sodium intakes, excessive water intake, or sodium retention [103–105]. To reduce the risk of patent ductus arteriosus, bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage, and long-term adverse outcomes a careful restriction of water is recommended, ensuring that physiological needs are met while avoiding dehydration [37, 107].

Chloride homeostasis also needs attention in premature infants because imbalanced sodium, potassium, and chloride intakes may promote metabolic acidosis or alkalosis [37]. Chloride requirements are generally considered similar to sodium requirements, but urine chloride excretion does not always parallel sodium excretion and chloride overload may affect acid-base status [108]. As for sodium, hidden or inadvertent chloride intakes are frequent because chloride is commonly combined with other nutrients, such as sodium, potassium, calcium, and AA, or with drugs like dopamine and dobutamine [108]. Excessive chloride intake increases acid load, and may result in metabolic acidosis. Thus, limiting chloride intakes and providing sodium and potassium intakes as organic phosphate or as sodium or potassium acetate/citrate in PN preparation might prevent hyperchloridemic metabolic acidosis [109–111].

Non-oliguric hyperkalemia (NHHK) in preterm neonates is defined as a plasma potassium level >6.5 mmol/L in the absence of acute renal failure [112]. Factors responsible for NOHK during the first week of life have extensively been described elsewhere [113, 114]. Other causes of early hyperkalemia may be birth traumas with hematomas/hemolysis, intracerebral hemorrhage, hypoxia, acidosis, or acute kidney injury [114].

Recent advances in neonatal medicine, such as widespread prenatal steroid use and early initiation of PN, have modified the course of postnatal fluid and electrolytes homeostasis [115–117]. By also reducing insensible water losses and optimizing early AA and energy intakes, it is possible to limit postnatal weight loss to 6–7% and to regain BW after 7 days on average in very preterm infants [19, 21, 56]. Such strategies have also improved the electrolyte homeostasis during the first 2 weeks of life. In particular, they have decreased dramatically the incidence of hypernatremia and NOHK [69, 109, 115]. Amino acid intakes ranging from 1.5 to 2.5 g/kg/day and given early after birth reduces catabolism and enhances the utilization of potassium [116]. If potassium and phosphate are not provided in sufficient amounts during the first days of life, early enhanced nutritional practices with sustained anabolic growth might put extremely preterm infants at risk of a refeeding like syndrome characterized by hypokalemia and hypophosphatemia [69–71]. Other causes of hypokalemia may be excessive gastrointestinal or renal losses, for instance, due to medications such as diuretics.

Postnatal Fluid and Electrolytes Monitoring

Rigorous monitoring of fluid and electrolyte homeostasis is required during the first week of life, especially in immature infants with high insensible water losses. Fluid and electrolyte balance should be assessed every 6–12 h during the first days of life by monitoring intakes, urinary output, body weight, plasma sodium concentration, and if needed acid-base status. Prevention of excessive insensible water losses is essential to maintain fluid and electrolyte homeostasis. Insensible water losses can be estimated by subtracting the weight change and the urinary output from the total fluid intakes. Additionally, excessive urine output (>5 mL/kg/h) may need to be replaced to avoid dehydration and hypernatremia. Excessive sodium and chloride intakes from medications should be controlled to avoid sodium and chloride overload. In very preterm infants, urine sodium fractional excretion is high during the first 1–2 weeks of life due to immature kidney functions. With improved tubular maturation (after the 1–2 second week of life), regular assessments of plasma and urine electrolyte concentrations may be helpful to adjust intakes.

Recommendations for Fluid and Electrolytes Supply

Current recommendations are to start with 60–80 mL/kg/day in older preterm infants or in infants with RDS and 80–100 mL/kg/day in more immature infants. To decrease transepidermal water losses, very premature and extremely premature infants should be kept within a double wall incubator and high-humidity environment [37]. Fluid supply should be increased progressively with careful monitoring of hydration status, allowing for a daily weight loss of 2–4%, with a final weight loss not exceeding 10%. A target fluid intake of between 140 and 160 mL/kg/day is estimated to maintain adequate fluid and electrolyte homeostasis and an appropriate weight gain (Table 7.1).

Sodium intake during the period of ECF contraction should be reduced to a minimum, allowing for a negative net sodium balance during the first 48 postnatal hours, until a weight loss of approximately 4–5% has occurred. Thereafter, intakes should be increased up to 3–5 mmol/kg/day (up to 7 mmol/kg/day in more immature infants). In clinical practice it is very difficult to completely avoid sodium administration on the 2 first days of life, as sodium is provided by other intakes (phosphate, and also other drugs). So the real challenge for the prescribing physician is to reduce as much as possible all inadvertent sodium intakes during the first 2 days of life.

Similar as for sodium, chloride should be supplied with caution during the period of ECF compartment contraction to allow for a negative chloride balance. Thereafter, 3–5 mmol/kg/day of chloride should be administered assuming the maintenance of a positive difference of 1–2 mmol/kg/day between the sum of sodium and potassium intakes and chloride intakes (Na + K–Cl = 1–2 mmol/kg/day). The use of sodium and/or potassium acetate or lactate (1–2 mmol/kg/day) instead of chloride in PN is helpful to prevent hyperchloridemia and metabolic acidosis (Table 7.1).

For potassium, an initial intake of around 1 mmol/kg/day is currently recommended on the first day of life to match higher protein and energy intakes from birth, providing that urine output is ascertained. Thereafter, intakes should be increased up to 2–3 mmol/kg/day in order to meet requirements for growth (Table 7.1).

Minerals: Calcium, Phosphorus, and Magnesium

Postnatal Mineral Metabolism

Fetal retention of calcium (Ca) and phosphorus (P) is dependent of growth and thus high during the last trimester of gestation. Estimating an average weight gain of 17 g/kg/d, the average Ca accretion is 3.4 mmol/kg/day and P accretion 2.6 mmol/kg/day [41]. Due to the interruption of placental transfer at birth and the high metabolic demand, a decrease in blood minerals concentrations may rapidly occur. In particular, it is well known that hypocalcemia may develop during the first days of life due to the relative immaturity of hormonal control (delayed parathyroid hormone increase) [1].

In addition to its implication in bone metabolism, phosphorus plays a critical role in energy metabolism. Hence, severe deficiency may induce several clinical disorders including muscle weakness, delay in weaning from respiratory support, glucose intolerance, and nosocomial infections [69, 70, 118, 119]. Extremely preterm or dysmature infants are particularly at risk for early hypophosphatemia due to their limited/depleted mineral stores and their very high growth rates. It has been shown that severe hypophosphatemia is potentiated by optimized AA intakes, warranting sufficient early supplies [69–71].

Reference values for hypophosphatemia differ between adults (1.0 mmol/L, 3 mg/dL) and preterm infants (1.6 mmol/L, 5 mg/dL) [1]. If laboratories use adult reference of plasma phosphate concentration, the diagnosis of hypophosphatemia may be easily missed with the risk of hypercalcemia, hypercalciuria, osteopenia, and nephrocalcinosis [120].

Optimal calcium to phosphorus ratio differs in parenteral and enteral nutrition due to the bypass of the gastrointestinal tract with PN, making Ca and P directly available for bone deposition [41]. Phosphorus retention is related not only to bone mineralization with a 1.7 calcium to phosphorus ratio (mmol/mmol) but also to lean body mass (LBM) accretion with the deposition of nearly 0.3 mmol (10 mg) of phosphorus for 1 g of protein accretion [120]. This needs to be taken into consideration when PN is initiated after birth. The recommended calcium to phosphorus ratio during PN is estimated to be between 0.8 and 1.0 (1–1.3 w/w) [41].

Fetal magnesium accretion is 0.12–0.20 mmol/kg/day (2.9–4.8 mg/kg/day), lower than that of calcium and phosphorous. The optimal magnesium requirements are not well defined for preterm infants on PN, and little attention is generally focused on postnatal magnesium homeostasis in neonates unless hypomagnesemia below 0.66 mmol/L (1.6 mg/dL) occurs in association with persistent and refractory hypocalcemia. Like for phosphorus, reference values of serum magnesium provided by laboratories are frequently the adult reference values (0.6–1.0 mmol/L). Studies in premature infants during the first 2 weeks of life show that their reference values are between 0.76 and 1.44 mmol/L, significantly higher than the adult reference range [121]. These studies also suggest that plasma magnesium concentrations are related to magnesium intakes, BW, and GA. Of note, magnesium concentrations are increased in cases of relative renal failure and in infants of mothers that have received magnesium sulfate before delivery. Antenatal magnesium sulfate administration is recommended in very preterm infants for the prevention of intraventricular hemorrhage [122–124]. In those infants, the postnatal magnesium concentrations are frequently between 1.4 and 1.8 mmol/L.

Minerals Sources

In contrast to enteral nutrition [41], calcium and phosphorus in PN are directly available for metabolism. Calcium may be provided in the form of calcium gluconate, calcium chloride, or calcium glycerophosphate. Calcium gluconate from glass containers should be avoided due to the risk of aluminum

contamination [125–127]. Calcium chloride is easy to use but its high chloride content needs to be considered in the electrolytes balance of the PN solution. Calcium glycerophosphate is not registered for use in PN, but may be prescribed from powdered anhydrous calcium glycerophosphate and reduces the risk of calcium-phosphate precipitations [1].

Phosphorus may be provided in the form of inorganic (sodium and potassium phosphate) or organic salts (glucose 1 phosphate, fructose 1–6 diphosphate, sodium glycerophosphate). Thus, phosphorus intake is also associated with sodium or potassium intake. Inorganic potassium phosphate induces a risk of precipitation that limits its use in PN. Organic phosphorus salts and especially disodium glucose-1-phosphate are widely used in PN solutions. However, their sodium content limits their utilization for premature infants, especially during the first days of life. To reduce sodium load and improve potassium intake during the early transition phase, the use of potassium salts over sodium salts should be considered.

Magnesium is generally provided as magnesium sulfate because magnesium chloride could induce anionic–cationic imbalance with the risk of metabolic acidosis.

Recommendation for Mineral Supply

Immediately after birth and during the first days of life, calcium supplementation should be given for preventing and treating early hypocalcemia. Early phosphorus administration is required to prevent severe hypophosphataemia, especially in SGA infants and very preterm infants receiving optimized AA and energy intake. In preterm infants exposed to antenatal magnesium sulfate, Mg intake should be adapted to postnatal blood concentrations.

In growing preterm infants on PN, the goal of mineral supply is to ensure optimal weight gain/growth and bone mineralization. Theoretical models of mineral accretion and average fetal weight gain have been used to estimate phosphorus and calcium requirements in parenterally fed preterm infants [41, 109]. Based on the most recent data in preterm infants receiving optimized PN, and in line with recent ESPGHAN guidelines, we recommend providing 0.8–2 mmol/kg/day of calcium (30–80 mg/kg/day), 1.0–2.0 mmol/kg/day of phosphorus (31–62 mg/kg/day), and 0.1–0.2 mmol/kg/day of magnesium (2.5–5.0 mg/kg/day) during the first days of life. Afterwards, intakes should increase progressively along with increases in macronutrients up to 1.6–3.5 mmol/kg/day for calcium (100–140 mg/kg/day) and phosphorus (77–108 mg/kg/day) and 0.2–0.3 mmol/kg/day for magnesium (5–7.5 mg/kg/day) (Table 7.1). In order to optimize the adequacy of mineral intakes in preterm infants on PN, regular monitoring of serum calcium, phosphate, magnesium, and alkaline phosphatase concentrations and urinary calcium and phosphate concentrations is recommended. The prevention of metabolic bone disease should be further assessed by periodic monitoring of vitamin D and may require bone mineral density assessment by appropriate techniques (e.g., Dual-energy X-ray absorptiometry).

Trace Elements

Trace elements are essential micronutrients involved in many metabolic processes. Their needs in premature infants during PN are not well defined, but Table 7.3 summarizes recent recommendations [40]. Micronutrient deficits are rare with current preparations of trace elements, except for zinc, which usually requires supplements, even during short-term PN. Zinc is required for the activity of a number of proteins, and it has a crucial role in maintaining quaternary structure stability of proteins and it participates in the regulation of gene expression, cell division, and growth [128]. Consequently, during anabolic processes resulting in higher protein synthesis, particularly after birth, the requirements of zinc increase. One recent study from Terrin et al. [129] has shown that in parenterally fed preterm infants zinc levels decrease from birth to day 28 of life and that there is a significant negative correlation between zinc levels and energy and protein intakes received during the first week of life. Zinc status should be periodically monitored in VLBW and ELBW infants on long-term PN and more often in those with high gastrointestinal fluid output (usually ileostomy losses), as they may have significantly higher zinc requirements.

In general, plasma levels of trace elements should be monitored during long-term PN (>4 weeks). Chromium and manganese frequently contaminates PN solution during preparation and additional supplementation is rarely necessary [130]. Excessive intakes are rare with current neonatal preparations, but in the case of cholestatic liver disease, copper and manganese toxicity may occur.

Table 7.3 Reasonable trace elements intakes for premature infants on parenteral nutrition

Iron	100–250 μg/kg/day
Zinc[a]	400–500 μg/kg/day
Copper[b]	40 μg/kg/day
Selenium	7 μg/kg/day
Chromium[c]	0.2 μg/kg/day
Molybdenum	1 μg/kg/day
Manganese[b]	0.5–1.0 μg/kg/day
Iodine	1–10 μg/kg/day

[a]Zinc to copper ratio should not exceed 20

[b]Copper and manganese intakes should be decreased in cholestatic liver disease

Commercial mixtures usually do not provide iron, but iron supplementation can usually be postponed during short-term PN (<3 weeks).

Vitamins

Vitamins are essential organic substances that humans cannot synthesize. Neonates have small vitamin stores and deficiencies may occur rapidly if not provided, especially in premature infants with high nutritional requirements and high metabolic rates. The optimal intake of vitamins for premature infants on PN is not well defined, and most studies were undertaken with commercial mixtures. Table 7.4 summarizes most recent recommendations [38].

There are few vitamin preparations designed for neonates on PN available on the market. They distinguish water-soluble and fat-soluble vitamins. Most pediatric vitamin formulations that are currently used in neonatal units are not designed for very preterm infants. Recent data suggest that additional intakes of fat-soluble vitamin A may provide some value for this patient population [38]. Additional vitamin E is however often not needed because the content of vitamin E is much higher in the newer composite LE than in soy-oil emulsions [38]. For premature infants, vitamin deficiencies are usually defined as below 200 µg/L for vitamin A and below 1 mg/dL for vitamin E, but for vitamin E a ratio below 0.8 mg/g of total lipid is also used. However, biological assessment of premature infant's vitamins status is only required in the case of long-term PN [38, 131].

Table 7.4 Reasonable vitamins supply for premature infants on parenteral nutrition

Water-soluble vitamins	
Thiamin (vitamin B1)	350–500 µg/kg/day
Riboflavin (vitamin B2)	150–200 µg/kg/day
Niacin	4–6.8 mg/kg/day
Pyridoxine (vitamin B6)	150–200 µg/kg/day
Folic acid	56 µg/kg/day
Cobalamin (vitamin B12)	0.3 µg/kg/day
Panthotenic acid	2.5 mg/kg/day
Biotin	5–8 µg/kg/day
Ascorbic acid (vitamin C)	15–25 mg/kg/day
Fat-soluble vitamins	
Vitamin A	700–1500 IU/kg/day
Vitamin D	80–400 IU/kg/day
Vitamin E[a]	2.8–3.5 IU/kg/day
Vitamin K[b]	10 µg/kg/day

1 mg niacin = 1 niacin equivalent (NE) = 60 mg tryptophan; 1 µg retinol equivalent = 3.33 IU vitamin A; 1 µg vitamin D (cholecalciferol) = 40 IU vitamin D (cholecalciferol); 1 mg tocopherol = 1 IU vitamin E

[a]Vitamin E need may be increased when using DHA and ARA parenterally

[b]0.5–1.0 mg vitamin K need to be given at birth

Fat-soluble vitamins are generally prepared in a 10% IVLE, whereas water-soluble vitamins are usually preserved as powder that needs to be dissolved by addition to IVLE, sterile water, or glucose solution. Several vitamins are light sensitive and need to be protected from light. Therefore, dilution of water- and fat-soluble vitamins in IVLE should be done to increase vitamin stability and to reduce peroxide load [38].

Individualized and Standardized PN Solutions

PN may be provided as separate infusions that merge at the infusion site (parallel infusions), as 2-in-1 solutions (carbohydrate, amino acids, and electrolytes) with the IVLE as a separate infusion, or with all components mixed in one bag (all-in-one solutions and 3-chamber bags). The development of ready-to-use industrially manufactured solutions, particularly multichamber bags, prolongs shelf life and minimizes the risks of inadvertent precipitations or contamination during compounding and storage [132].

Standard PN bags are formulated to meet the need of the majority of patients within an age or weight group, whereas individual tailored PN formulation are made to cover the specific requirements of an individual patient [29]. Both of these methods have advantages and disadvantages associated with their use. The main advantage of individualized solutions is that each solution is formulated for one individual patient and that they can be modified when the patient's nutritional needs and metabolic, electrolytes, or clinical status change. However, in premature infants, the clinical course may change rapidly, also necessitating adjustments of the individualized solution. The advantage of standardized solutions is that they include all the essential nutrients in fixed amounts, which eliminates the chances of inadvertent omissions or overload if they are used appropriately. Due to their lack of patient specificity some minimal adjustments are commonly needed, particularly during the first days of life.

Several studies suggest that the use of standardized ready-to-go PN solutions improve nutrition therapy in neonates [21, 22, 55, 56, 115, 133, 134], and standardized versus individualized PN is thus recommended for stable premature infants [29]. It is important to remember that vitamins and trace elements need to be added before administration, preferably by the pharmacy department.

Conclusion

Prematurity occurs during a critical period of development, and optimal nutritional support represents a major challenge for all healthcare providers involved in the nutritional care of

these infants. This chapter gives a comprehensive overview of nutrient requirements in preterm infants and provides an update on best practice recommendations.

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Infectious Esophagitis

8

Salvatore Oliva, Sara Isoldi, and Salvatore Cucchiara

Introduction

Infectious esophagitis is relatively rare in an immunocompetent host and usually is an indicator of a primary or secondary immunodeficiency. In immunocompetent children, infectious esophagitis frequently is associated with conditions that compromise esophageal defense mechanisms [1–3]. The spectrum of esophageal infections has changed over the past few decades. Infectious esophagitis was rare before the advent of acquired immune deficiency syndrome (AIDS) and posttransplant immunosuppressive treatment regimens [1].

Though immunosuppression, in general, can result in infectious esophagitis, infections are most likely to occur in children with HIV infection, during chemoradiotherapy for hematologic malignancies and after solid organ and hematopoietic stem cell transplantation [3–7]. Infectious esophagitis has also been reported in 3% of patients with ataxia telangiectasia [8].

The risk of opportunistic infectious esophagitis in HIV is related to the CD4 count with patients noncompliant to highly active retroviral therapy (HAART) more likely to be infected [9]. Chemotherapy and radiation may predispose to esophageal infections due to the immunosuppression as well as the direct cytotoxic effects on the mucosal barrier. Hematological malignancies are more likely to be associated with infectious esophagitis than solid tumors, though the risk is attenuated with routine antimicrobial prophylaxis [10]. The risk of esophageal infections in bone marrow transplantation is higher in allogeneic than autologous transplants.

In an immunocompetent individual, impaired esophageal clearance of swallowed organisms may foster a permissive environment for the development of esophageal infections. These would include impaired saliva production, altered esophageal motility contributing to stasis, and gastric hypochlorhydria. Injury to the esophageal mucosa either from inflammation or endoscopic procedures may facilitate infection in certain instances [3, 4]. The most common cause of infectious esophagitis is Candida, which is a symbiont of the esophagus, but in case of impairment of host defenses, it can proliferate and develop adhesive plaques [11]. The second most common cause is cytomegalovirus (CMV), followed by human immunodeficiency virus (HIV), tuberculosis, varicella zoster virus (VZV), and human papilloma virus (HPV) [11].

Clinical Features

The clinical approach to evaluate a suspected infectious esophagitis is guided by the presence of any underlying immunosuppression, the presenting symptoms, and the physical findings. Pathologic gastroesophageal reflux is the most common cause of esophagitis in children. In previously healthy children, esophageal symptoms are likely to be caused by reflux esophagitis, whereas in immunocompromised patients, the physician needs to rule out infectious esophagitis. Secondary bacterial or fungal infections can be present in reflux esophagitis and Chagas disease, especially with severe inflammation and obstruction. Absence of reflux symptoms (long-standing heartburn, a water brash taste in the mouth, vomiting, spitting up in infants, pillow wetting, or coughing) does exclude reflux esophagitis. Achalasia, diffuse esophageal spasm, foreign body impaction, and mediastinal or retropharyngeal abscesses can cause esophageal symptoms and may result in secondary infection [2].

Patients with infectious esophagitis can present with esophageal, abdominal, or systemic symptoms; however, patients are often asymptomatic. Odynophagia and dysphagia are the most common symptoms of esophagitis, but they may not be apparent in small children [5]. In adults, only 59–79% of patients with documented infectious esophagitis

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had these symptoms [4]. Odynophagia is usually indicative of underlying esophageal ulceration. The absolute CD4 count stratifies the risk of an opportunistic infection in HIV and coinfections may occur, especially with profound immunosuppression [12].

Diagnosis

Establishing a specific diagnosis is essential for managing of infectious esophagitis, particularly because fungal and bacterial superinfections occur commonly in viral esophagitis.

A key diagnostic challenge in infectious esophagitis is ascertaining the role of an isolated microorganism in disease causation as many purported pathogens are commensals that may be found even in healthy individuals [1]. Colonization of the oral cavity facilitates colonization of the esophagus following deglutition. Hence diagnosis of infection requires corroborative endoscopic and histological findings. The use of viral culture and polymerase chain reaction (PCR) may increase the diagnostic yield, however decreasing specificity partly due to contamination from latently infected cells. Serologic testing has little role in the evaluation of esophageal infection [1–3].

Esophagoscopy, Biopsy, and Brushing

The technique for performing endoscopy with videoendoscopes miniaturized to a diameter of 4.8 mm has made the procedure far more tolerable in immunocompromised patients, requiring less sedation and anesthesia and allowing the procedure to be performed even in newborns. These new instruments have biopsy capabilities comparable to older, wider endoscopes [2].

Characteristic macroscopic lesions are associated with some infectious agents. Macroscopic appearances overlap considerably, however, and histopathologic or immunohistochemical analysis (or both) of endoscopic and brush biopsy specimens is essential for diagnosis. Endoscopic biopsy specimens should be obtained from the edge and the base of the lesions. In CMV infection, specimens from the edge do not yield diagnostic information [13]. The pathologist should be alerted to the possibility of fungal, viral, and polymicrobial infections. Appropriate fixatives should be used for routine hematoxylin and eosin stain, Gram stain, and special stains for fungi and bacteria such as *Mycobacterium*. Immunohistochemical studies and DNA hybridization techniques often are required to establish the diagnosis [1].

Fungal Esophagitis

Candida

Candida is the most common cause of infective esophagitis [14], and immunosuppressed patients are prone to this infection. Candida esophagitis is common in HIV-infected patients, in whom it tends to occur when CD4 counts fall below 200 µl. Treatments with broad-spectrum antibiotics, acid-suppressive therapy, and inhaled corticosteroids are also risk factors [15–17]. In apparently immunocompetent individuals, predisposing medical conditions or risk factors are often identified [18]. The most common risk factors identified among studies are corticosteroids, antibiotics, and malignancy. Similarly, the use of proton pump inhibitors (PPI) has also been associated to the development of candida esophagitis; Hoversten et al. reported that PPI contributed to the development of infectious esophagitis in 63–81% of immunocompetent patients [19]. The causative organism is almost always *Candida albicans*, although other species are occasionally found [20]. Differentiation between different species is of clinical significance, as *glabrata* species usually exhibit azole resistance [11].

Patients present with dysphagia, odynophagia, and/or retrosternal chest pain. Esophageal candidiasis may be the initial presentation of disseminated disease in an immunocompromised patient. The majority of patients, particularly those who are immunocompromised, also have concomitant oropharyngeal candidiasis [20]. Nearly all patients with AIDS, oral candidiasis, and odynophagia have endoscopic evidence of esophageal candidiasis [5, 21, 22]. However, some of these patients ultimately proven to have esophagitis have no signs or symptoms at presentation. Children who develop esophageal candidiasis, despite being treated with HAART, are less likely to have typical symptoms (e.g., odynophagia and retrosternal pain) or to have concomitant oropharyngeal candidiasis [12]. Candida esophagitis can be distinguished into "acute infection," which is typical of severely immunocompromised patients and generally evolves into patient exitus; "subacute infection," which is generally related to complications such as esophageal pseudodiverticulum or stricture; and "chronic infection," which is typical of immunocompromised children [23].

A specific etiological diagnosis is established by endoscopy with or without biopsy or brushings. Endoscopy reveals the presence of characteristic confluent yellow-white plaques overlying and adherent to an erythematous mucosa. Remarkably, the presence of ulceration is unusual and should prompt further evaluation for an alternative etiology. Endoscopic findings without biopsy have been reported to

have a sensitivity and specificity of 100% and 83%, respectively, for a diagnosis of candida esophagitis [24].

The histological examination shows active esophagitis with budding spores and pseudohyphae within squamous debris, ulcer slough, and fibrino-purulent exudate. Invasion of mucosal and submucosal blood vessels is sometimes a prominent feature of invasive candidiasis [25]. *Candida* species are commensal organisms of the gastrointestinal tract and colonize the esophagus in about one-fifth of healthy adults, making histological evidence of fungal invasion into tissue or ulcer slough important [16]. Parakeratosis with neutrophils may call attention to the presence of *Candida*.

Brushings may be obtained and stained with Gomori silver or periodic acid-Schiff stains. Fungal culture is not performed routinely as it is generally not useful except in defining the species and drug sensitivities, especially in treatment resistant cases [25].

Candida can colonize preexisting ulcers or damaged mucosa of any etiology, and the pathologist should consider the possibility of dual pathology.

Expert consensus guidelines recommend systemic therapy with newer azole medications (fluconazole, itraconazole solution, or voriconazole) for esophageal candidiasis. Topical therapy may produce an initial response, but early treatment failures are common. Oral fluconazole 200–400 mg (3–6 mg/kg) daily for 14–21 days is recommended. If oral therapy cannot be tolerated, intravenous fluconazole 400 mg daily, amphotericin B, or an echinocandin, such as caspofungin, micafungin, or anidulafungin may be used [23, 26, 27]. Oral fluconazole and itraconazole suspension seem comparable in efficacy for initial therapy, and some patients whose disease fails to respond to fluconazole may see improvement with subsequent itraconazole therapy [28]. Although voriconazole has efficacy similar to that of fluconazole, it is associated with a higher rate of adverse events [29].

Other Causes of Fungal Esophagitis

Cases of esophagitis caused by *Aspergillus*, *Blastomyces*, *Cryptococcus*, and *Histoplasma* spp. have been described. Unlike Candida, these are not commensals and are acquired by significantly immunocompromised individuals from the environment. Primary infection of the esophagus is very unusual and has been described in immunocompromised patients. *Aspergillus* infection occurs as a result of contiguous spread from mediastinal infection [30, 31]. *Blastomyces* and *Histoplasma* infect the esophagus from a concomitant pulmonary infection or from disseminated infection [1]. Mediastinal fibrosis with esophageal obstruction and esophageal fistula may occur with histoplasmosis [31].

Optimal therapy has not been established, but systemic therapy, as for other manifestations of invasive infection with these organisms, has been successful. Currently, this approach would involve intravenous or oral azole agents or an amphotericin B preparation. Echinocandins (micafungin, caspofungin, or anidulafungin) may be useful for *Aspergillus* or *Histoplasma* infections, but they have no activity against cryptococci.

Viral Esophagitis

Herpes Simplex Virus

Herpes simplex virus (HSV) esophagitis is primarily a disease of immunocompromised patients and occurs most commonly in patients with solid organ and bone marrow transplants [32–34]. These patients may have life-threatening disseminated infection at the time of diagnosis [32]. It has also been reported in the setting of acute rejection [34]. In contrast to transplant recipients, it accounts for only 3–5% of esophagitis in HIV patients [35–37]. Most infections in immunocompromised patients probably represent viral reactivation as these patients have higher baseline seroprevalence rates. HSV esophagitis can occasionally occur in subjects with normal immune function, who usually have a self-limiting infection that resolves spontaneously within 1–2 weeks [38, 39].

Although the esophagus is the most frequent site of gastrointestinal involvement, HSV esophagitis is uncommon. The vast majority of documented cases are due to HSV-1, though HSV-2 esophagitis from heterosexual oro-genital contact in an immunocompetent patient has been described [40]. Interestingly, recent case reports and series report the association of eosinophilic esophagitis with HSV esophagitis, especially in the pediatric age population [41, 42]. However, the relationship between them has not been cleared yet.

Symptoms of HSV esophagitis are similar in both immunocompetent and immunocompromised patients. In the immunocompetent it is characterized by the acute onset of odynophagia, while 60–76% of patients exhibit retrosternal chest pain or heartburn [38, 43]. This may be associated with fever and systemic manifestations. Unlike in Candida, where oral disease is present in the majority of patients, coexisting herpes labialis and oropharyngeal ulcers are only seen in about one-fourth of patients [4, 38]. Symptoms may be more severe in immunocompromised patients with bleeding, per-

foration, tracheoesophageal fistula, and necrotizing esophagitis having been reported [44–46].

Endoscopic findings include nonspecific erosive esophagitis and discrete or coalescent superficial ulcers with an exudate. Vesicles that are the earliest manifestations are rarely seen. They coalesce to form ulcers often with normal intervening mucosa. Multiple esophageal ulcers are the commonest endoscopic finding seen in 59–86% of HSV patients, but these are nonspecific [38, 43]. The ulcers are usually small and discrete or occasionally confluent. The ulcers are "volcano-like" in appearance, in contrast to CMV ulcers which are linear or longitudinal and deeper. In addition to ulcers, friable mucosa and white exudates are commonly seen on endoscopy in HSV esophagitis. The distal esophagus is the commonest site of involvement; the entire esophagus may be involved in 15% of patients [38].

The diagnosis is usually based on a combination of histological findings and viral isolation from culture. Biopsies from the edge of the ulcers provide the highest diagnostic yield as the base of the ulcers often lack epithelial cells. Ulceration with neutrophils and an inflammatory exudate may be seen. Squamous cells may show viral cytopathic effects, including multinucleation, groundglass nuclei, and dense eosinophilic inclusions with a thickened nuclear membrane and a clear halo (Cowdry type A inclusion bodies). However, viral inclusions and multinucleated cells are not always identifiable in endoscopic biopsies [32] and may also be seen in other viral infections such as those caused by CMV and VZV. Aggregates of macrophages with convoluted nuclei have been identified adjacent to infected epithelium and may make the pathologist suspect herpes virus infection and prompt further investigation [47]. HSV isolation in the absence of histological findings is of questionable significance as it may represent asymptomatic viral shedding.

Acyclovir for 14–21 days has been advocated in the treatment of immunocompromised individuals with the use of intravenous preparations in those unable to swallow. In contrast to immunocompromised patients, HSV esophagitis in the immunocompetent is an indolent but usually self-limiting disease. The value of treatment with acyclovir is uncertain [43]. While case reports suggest therapeutic benefit in hastening illness resolution [39], the relative rarity of the condition precludes any randomized controlled trials, and spontaneous resolution usually occurs within 1–2 weeks. A search should be made for any underlying immunosuppressive illness in patients presenting with HSV esophagitis.

CMV

CMV is a significant pathogen in the immunocompromised subject, usually occurring in patients with AIDS [24], transplant recipients, malignancies, and those receiving immunosuppressive medications. These patients frequently have multiorgan involvement, while CMV infection is usually subclinical and asymptomatic in immunocompetent patients [48]. Indeed, serious symptomatic CMV esophagitis in immunocompetent patients is rare [49].

The most common gastrointestinal involvement in patients with CMV infection is colitis, followed by esophagitis [50].

Patients with CMV esophagitis clinically present with dysphagia, odynophagia, or nonspecific symptoms such as nausea, vomiting, abdominal pain, anorexia, and fever that reflect multiorgan or systemic involvement. Thrombocytopenia and leucopenia may be present but are not invariable.

Endoscopy may reveal variable findings from esophageal erosions to deep ulcers, located in the mid or distal esophagus with a halo of edema. In the setting of hematopoietic stem cell transplantation, they may be macroscopically confused with graft-versus-host disease. Stricture formation is relatively uncommon despite the occurrence of deep CMV ulceration [51].

Diagnosis needs a combination of histology and of demonstration of CMV in tissue specimens. The histological hallmark of CMV is the presence of cytomegalic cells on hematoxylin and eosin staining of mucosal biopsy. The infected cells are enlarged and contain Cowdry type A intranuclear inclusions with a surrounding halo ("owl's eye" inclusion bodies). The presence of "owl's eye" inclusions is highly specific, although not sensitive for the determination of CMV organ involvement. Hematoxylin and eosin staining have comparable sensitivity and specificity to immunohistochemical staining with monoclonal antibodies but at a lower cost [52]. Since CMV has a predilection for fibroblasts and not squamous epithelial cells, biopsies should be obtained from the base of ulcers. The use of CMV DNA PCR on mucosal biopsy specimens increases the detection of CMV, but only a fraction of these patients has typical histological changes [53]: noticeably, this increased yield with PCR may be due to contamination from latently infected cells. Likewise, the late CMV antigen assay has 89–100% sensitivity for CMV viremia but are not predictive for CMV disease [54, 55]. Conversely, CMV disease as detected on histology may be present even in the absence of CMV detection in the blood.

IV ganciclovir or foscarnet are first-line treatment for CMV disease. In patients with severe clinical symptoms and high risk of developing CMV esophagitis (CD4 count <50), treatment should be initiated before pathological confirmation. Oral valganciclovir has been shown to be non-inferior to IV ganciclovir for treating CMV in certain solid organ transplantation recipients [56]. However, IV ganciclovir is preferred in patients who do not tolerate oral treatment and those with life-threatening CMV disease. The use of foscar-

net has been limited by its nephrotoxicity. The role of maintenance treatment is not well defined. Reduction in immunosuppression should be individualized but considered in transplant recipients with CMV disease.

Other Viral Infections

VZV is rarely associated with esophagitis in severely immunocompromised patients. The typical cutaneous vesicular eruptions are usually present when esophagitis occurs. Esophageal VZV may be a harbinger of disseminated VZV [57]. The endoscopic appearance may be variable with vesicles and ulcers seen [58]. Esophago-bronchial fistula has been reported to occur in VZV infection in an AIDS patient [59]. Biopsies reveal ballooning degeneration, multinucleated giant cells, and intranuclear inclusion bodies similar to HSV, and viral culture is needed to distinguish the two viruses. Though VZV esophagitis is self-limited in immunocompetent patients, it is typically treated with acyclovir for routine and foscarnet for resistant cases.

HPV infection is generally associated to genital warts and cervical cancer. Endoscopic esophageal involvement include ulceration, hyperkeratosis, and papillomas [60]. Histology reveal the presence of koilocytosis, perinuclear clearing, and giant multinucleated cells [11]. HPV has been recognized as a risk factor of development of esophageal squamous papillomas, which are benign lesions of the esophagus [60]. Inconclusive data are available regarding the role of HPV in the development of esophageal squamous cell carcinoma [61]. A single treatment of liquid nitrogen cryotherapy has proven to be effective in treating diffuse esophageal papillomatosis [62].

Esophageal ulcerations have been very rarely reported in Epstein-Barr virus infection in both immunocompetent and immunocompromised patients [63]. The ulcers are deep, linear, and involve the mid-esophagus. Koilocytosis, epithelial thickening, and cell multinucleation are seen on biopsy. The number of patients is too small to draw any firm conclusion on treatment indications [64].

Bacterial Esophagitis

Bacterial infection is a rare cause of esophagitis. It typically occurs in immunocompromised hosts such as those with hematologic malignancies with neutropenia, bone marrow transplantation, diabetic ketoacidosis, and steroid therapy [65, 66]. It is usually polymicrobial and derived from oral flora (i.e., *Streptococcus viridians*, *Staphylococci*, and *Bacillus* spp). Esophagitis in frequently associated with bacteremia; hence, blood cultures always should be performed.

The diagnosis is made by demonstrating bacterial clusters on Gram stain with evidence of subepithelial bacterial invasion on endoscopic biopsies [67]. Treatment is with broad-spectrum antibiotics. Lack of response to appropriate therapy may indicate concomitant superinfection by other organisms or resistance to the drugs used. Repeat endoscopy is indicated for documenting eradication of infection.

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Eosinophilic Esophagitis

9

Mason Nistel and Glenn T. Furuta

Introduction

During the last 20 years, an increasing number of clinical experiences and research studies identified the clinicopathological findings associated with children and adults who experienced unexplained feeding problems, recalcitrant gastroesophageal reflux disease (GERD) symptoms, dysphagia, and food impaction. These patients did not respond to GERD treatments and demonstrated distinct endoscopic abnormalities such as white exudates, furrows, and long segment narrowing, as well as dense esophageal mucosal eosinophilia that extended along the length of the esophagus. Other causes notwithstanding, these patients were found to have a chronic esophagitis of allergic etiology, and were responsive to elimination of dietary allergens or topically delivered steroids [1]. An increasing body of evidence demonstrates that the underlying etiology of these patients' symptoms, endoscopic findings, and eosinophilia are due to an overactive Th2 cytokine pathway [2]. Taken together, characterization and refinement of this constellation of findings led to the consensus diagnostic criteria defining eosinophilic esophagitis (EoE) as a chronic allergic inflammatory disease of the esophagus.

Historical Context

In the 1970s, use of endoscopy and mucosal biopsies in adults brought the initial link of esophageal eosinophilia with GERD. In 1993 and 1994, two studies provided comprehensive descriptions of adults with food impactions and/or dysphagia who were also found to have dense esophageal eosinophilia [3, 4]. Patients were distinct from those with GERD as they did not respond to acid blockade or had normal pH monitoring of the distal esophagus and were eventually termed to have eosinophilic esophagitis (originally EE but changed to EoE to eliminate confusion with erosive esophagitis). During the next decades, basic investigations identified an allergic pathogenesis, and clinical studies developed treatment algorithms using food eliminations and topical steroids [5–7].

Diagnostic Criteria

Between 2007 and 2019, collaborative efforts developed and revised the EoE diagnostic criteria four times [1, 8–10]. EoE is currently defined as a chronic, allergic, inflammatory esophageal disease characterized by symptoms of esophageal dysfunction and dense esophageal eosinophilia for which other causes have been ruled out. The 2007 consensus diagnostic guidelines were established by a multidisciplinary group of 31 physicians and defined EoE as a clinicopathologic disease of allergic nature thus distinguishing it from the more common GERD. At that time, diagnostic criteria required symptoms related to esophageal dysfunction such as dysphagia, food impaction, feeding intolerance/difficulties, and reflux, histologic findings of esophageal mucosal inflammation with >15 eosinophils/HPF, and a trial of proton pump inhibitors, a key element in ruling out GERD [8]. Updated consensus guidelines were published in 2011 that added chronicity to the definition, as well as inclusion of EoE pathogenesis via an immune/antigen mediated pathway in the setting of genetic predisposition. This position statement was the first to address a new phenotype of esophageal eosinophilia that responded to high-dose proton pump inhibitor (PPI), termed PPI-responsive esophageal eosinophilia (PPI-REE). Patients with PPI-REE exhibited symptoms of

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esophageal dysfunction with pathology that demonstrated >15 eosinophils/HPF, normal pH monitoring, and remission on PPI therapy only [1]. In the 2013 consensus statement, the recommendation of 2–4 biopsies in both the proximal and distal esophagus was made along with a description of common EoE endoscopic findings (EREFS) [9]. Then, in 2018, the AGREE (*A* working *G*roup on proton pump inhibitor *R*esponsive *E*sophageal *E*osinophilia) meeting removed the PPI trial from the EoE diagnostic criteria, and suggested that patients with PPI-REE likely represented a subset of EoE [10]. This change was supported by similar RNA transcriptome data between patients with EoE and PPI-REE [11].

Other studies demonstrated that PPI-REE symptoms recurred upon discontinuation of PPI and subsequently responded effectively to dietary elimination therapy or topical steroids [12, 13]. The AGREE guidelines state that EoE cannot be definitively ruled out in patients who are on PPI at the time of endoscopy [10]. Current diagnostic criteria include symptoms of esophageal dysfunction including food impaction, dysphagia, food refusal, reflux symptoms, chest pain, and histologic findings of >15 eosinophils/HPF with exclusion of other causes of esophageal eosinophilia. Aside from EoE and GERD, other clinical causes of esophageal eosinophilia are rare and can often be distinguished by specific presentation or histology (Table 9.1). Providers should have heightened suspicion for EoE in patients with co-occurring atopic conditions such as asthma, eczema, food allergies, and family history of food impactions, esophageal dilation, EoE, or dysphagia. Except for diminished growth, no physical exam findings are pathognomonic of EoE [9].

Factors Complicating EoE Diagnosis

Multiple factors can complicate making a definitive diagnosis of EoE including treatment with systemic steroids or topical steroids for another indication (asthma, allergies) at the time of a diagnostic endoscopy, seasonality, inadequate mucosal sampling with too few biopsies, or biopsies obtained from a patient with longstanding mucosal inflammation. Given the frequent coexistence of asthma and other atopic conditions with EoE, clinical experiences identified that some patients with comorbid allergic diseases may be treated with systemic or topical corticosteroids that could partially reduce esophageal eosinophilia found at the time of a diagnostic biopsy [1]. Patients may present with features highly suspicious of EoE, such as recurrent food impactions, endoscopic findings or esophageal rings, or family history of unexplained esophageal strictures, but demonstrate mucosal eosinophilia less than the 15 eosinophil/HPF threshold. In this case, consideration, in association with the patient and family, should ensue regarding a limited medication trial to assess for symptomatic response and eventual repeat endoscopic assessment.

Table 9.1 Differential diagnosis for esophageal eosinophilia

Gastroesophageal reflux disease
Eosinophilic esophagitis
Eosinophilic gastrointestinal disorders
Infection (parasitic or fungal)
Crohn's disease
Celiac disease
Hypereosinophilic syndromes
Drug hypersensitivity reactions
Pill esophagitis
Vasculitis
Achalasia
Graft-versus-host disease
Oral immunotherapy
Pemphigus

Liacouras et al. [1]

The seasonal timing of a mucosal biopsy may also influence mucosal eosinophilia. For instance, clinical studies have demonstrated that biopsies obtained in the fall and spring have increased esophageal eosinophilia compared to winter and summer, likely due to differences in pollen exposure. A retrospective analysis of 127 adults with EoE demonstrated seasonal variation in the frequency of EoE diagnosis that correlated with pollen levels [14]. Seasonality was also identified in a pediatric population through a retrospective review of 1,180 patients, 32 of whom were found to have definitive seasonal variation of their esophageal eosinophilia [15]. Thus, extent of eosinophilia in some patients may depend on the season, and the diagnosis could be missed if biopsies were obtained outside of an allergy season.

The diagnosis may also be missed because of the patchy nature of EoE and the resultant inadequate sampling of an affected area. Both adult and pediatric studies support obtaining mucosal biopsies in at least two different locations with a total of six samples in order to approach a sensitivity greater than 90% [16–18].

The natural history of EoE continues to evolve, but multiple studies indicate that a number of different phenotypes exist. An acute inflammatory pattern is well described in pediatrics to be exhibited by white exudates and linear furrows. In adult patients, the chronicity of the disease is demonstrated by the strictures and fibrosis [19, 20]. A prospective study of 70 adults included functional luminal-imaging probe (FLIP) evaluation during endoscopy to assess esophageal distensibility compared to level of epithelial eosinophilia and risk of food impaction. Patients with previous food impactions had significantly lower distensibility; however, no correlation to mean eosinophil density was found [21]. In this subset of patients with longstanding inflammation, biopsies may not meet the >15 eosinophil/HPF histologic threshold despite advanced symptomatic disease.

Clinical Presentation

Presenting Symptoms

Presenting symptoms of EoE vary based on age. Differences in reported symptoms may be due to the difficulty of younger patients to articulate their experiences. In the toddler age group, the most frequent symptoms are associated with GERD such as vomiting, regurgitation, as well as those related to feeding dysfunction or meal time problems [22]. Meal time behaviors may be nonspecific and include tantrums, spitting food out, poor appetite, and slow eating, all of which can be upsetting to families [22]. In addition to these symptoms, school age patients may also present with abdominal pain, reflux, and dysphagia [10, 23, 24].

Compensatory behaviors frequently develop and may be viewed as behavioral issues rather than as problems reflective of an underlying disease [22]. These behaviors are described with the acronym IMPACT: imbibe with meals, modify food (cutting into small pieces), prolong meal times, avoid hard textures, chew excessively, and turn away tablets/pills [25]. With these modifications, patients rarely progress to the point of frank failure to thrive or nutritional deficiency [22].

Adolescent and adult patients most commonly present with symptoms of dysphagia and food impaction and can also present with chest pain and heartburn [1]. Dysphagia may be described as the feeling that food is getting stuck or traveling slowly as well as gagging on food. Adult patients with EoE also can develop worsening of gastrointestinal symptoms with alcohol intake [26]. A novel syndrome found in some patients with EoE is referred to as food-induced immediate response of the esophagus (FIRE). This was recently described as an unpleasant or painful retrosternal sensation that occurs immediately and reproducibly after ingestion of a specific food or beverage [27]. Symptom latency is generally less than 5 minutes following the ingestion of the food and lasts less than 30 minutes with high symptom intensity. Patients clearly differentiate this phenomenon from EoE-related dysphagia or food impaction, and the most frequently implicated triggers include milk, wheat, nuts, fruits, wine, and beer. Rare presentation of EoE includes esophageal rupture or Boerhaave's syndrome [28].

Patients diagnosed with EoE have a high likelihood of concomitant atopic history [29]. A large retrospective analysis compared the risk of 7,722 EoE patients having atopic conditions to controls without EoE. Results were notable for risk ratios of 2.24, 2.19, 1.53, and 17.05 for allergic rhinitis, asthma, atopic dermatitis, and food allergies, respectively, in patients with EoE [30]. Similarly, a prospective study of 70 pediatric patients, 33 with EoE and 37 healthy controls, evaluated responses to methacholine challenge [31]. In the EoE cohort, 33% of patients had significant increase in airway hyperresponsiveness compared to 11% of controls. EoE occurs in patients with IgE-mediated food allergies at a rate of 4.7% compared to the lower rate in the general population of 0.4% [2].

Natural History

The natural history of EoE has yet to be defined, but several possibilities are proposed. A prospective study of 185 patients (88 children) identified 3 distinct EoE endotypes based on peak eosinophil count, a distinct set of genes identified in the EoE diagnostic panel (EDP), the EoE histologic scoring system (EoE-HSS), and the EoE endoscopic reference score (EREFS) [32]. Endotype 1 is the mildest and most steroid-sensitive. Endotype 2 has substantial inflammatory changes, demonstrates a Th-2 immune response, and is often steroid refractory. Endotype 3 is most common in adult-onset EoE, shows the lowest expression of epithelial differentiation genes, and the most severe esophageal narrowing, endoscopic, and histologic changes. Another consideration is that untreated EoE patients may progress from an acute inflammatory to a fibrotic pattern. During the acute stage, patients exhibit exudates and edema with reflux-like symptoms, whereas the fibrotic pattern is represented by strictures, dysphagia, and food impactions. A retrospective study of 379 children and adults with EoE determined that for each decade of life, the likelihood of developing a fibrostenotic phenotype more than doubled (OR 2.14) [33]. Similarly, in a retrospective review of 200 adult patients with EoE, a diagnostic delay of 0–2 years corresponded to a stricture prevalence of 17.8% compared to a diagnostic delay of >20 years which identified a stricture prevalence of >70.8% [20].

Endoscopic Findings

Esophageal endoscopic findings associated with EoE include loss of vascular pattern (LOVP), rings, white exudates, linear furrowing, strictures, and mucosal fragility or crepe paper esophagus, a finding defined by the creation of longitudinal rents following passage of the endoscope (Fig. 9.1 and Table 9.2). Each of these represent different inflammatory states [34]. For instance, LOVP and furrows reflect edema and exudates represent eosinophilic pus. In contrast, rings, strictures, and longitudinal rents likely reflect evidence of chronic inflammation. A 2012 meta-analysis of 4,678 adults and children showed that the most common endoscopic finding was linear furrowing (48%) followed by esophageal rings (44%) and LOVP (41%) [35]. When evaluated by age, rings and strictures were more prevalent in adults while chil-

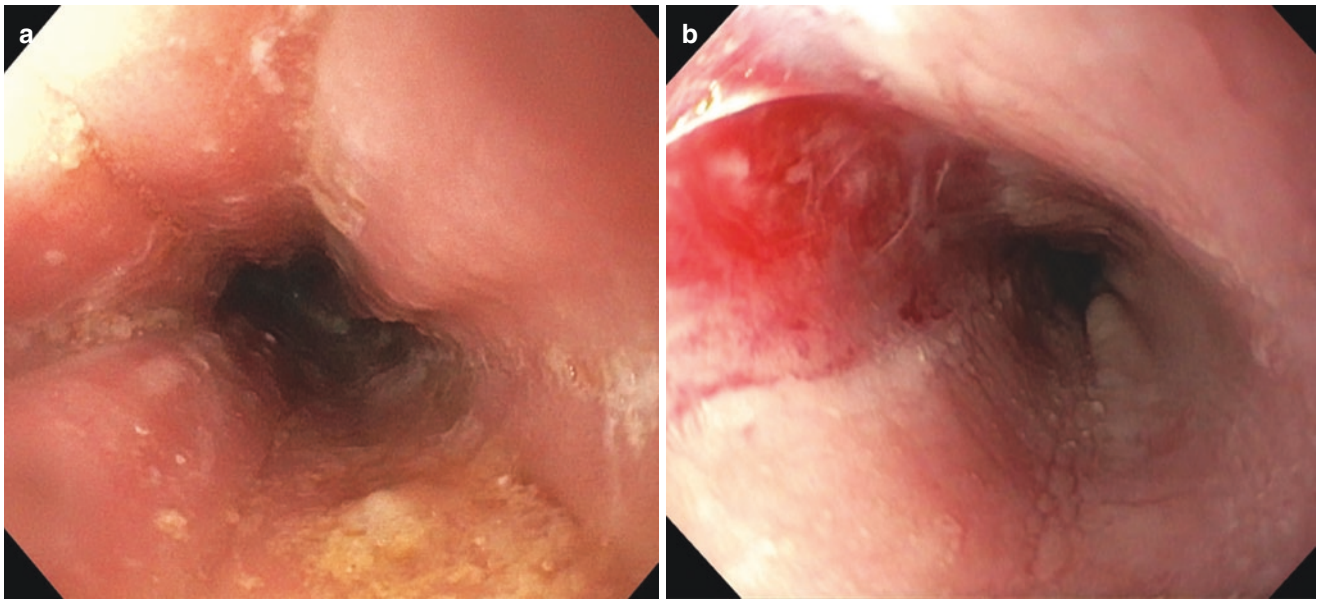

Fig. 9.1 Endoscopic findings associated with eosinophilic esophagitis include (**a**). loss of vascular pattern, white exudates, and furrowing (EREFS score-E1E1F1 score 3) in a 13-year-old with history of a food impaction; (**b**). longitudinal rent and furrows in a 14-year-old observed following passage of the endoscope

Table 9.2 Endoscopic findings associated with eosinophilic esophagitis

Whitish exudates
Loss of vascular pattern
Furrowing
Rings
Long segment narrowing
Strictures
Crepe paper esophagus—rent formation
Esophageal pull (tug) sign

Kim et al. [35] and Dellon et al. [36]

dren present with significantly increased prevalence of white plaques (36%) and LOVP (58%). Importantly, 17% of cases appeared normal, emphasizing the necessity of obtaining mucosal biopsies when EoE is suspected regardless of mucosal appearance. Sensitivity, specificity, and positive predictive values of endoscopic signs are not presently sufficient for diagnosis; however, prevalence is high of at least one endoscopic finding in patients with EoE [35].

An endoscopic phenomenon referred to as the esophageal pull (tug) sign provides a strong independent predictor for diagnosis [36]. This sign is characterized by inability to completely close endoscopic biopsy forceps, prolonged tenting of the esophageal mucosa when the forceps is retrieved, and more force than usual needed to retrieve the forceps or the need of a "tug."

A validated EoE endoscopic scoring system called the EoE Reference Score (EREFS) uses the findings of exudates, rings, edema, furrows, and strictures to develop a numerical score [37]. EREFS is effective in providing valuable diagnostic information and monitoring in both adults and children. Validation of the score revealed good interobserver agreement of 71–81% for rings, furrows, exudates, and edema in addition to 79% and 92% for stricture and crepe paper esophagus, respectively [37]. A prospective pediatric study of 371 patients evaluated EREFS in 192 diagnostic endoscopies and 229 post-treatment endoscopies [38]. Outcomes of the study showed a strong correlation between composite EREFS score and the degree of eosinophilia and no significant correlations with individual endoscopic findings. The score was also able to differentiate newly diagnosed EoE patients from controls, and active EoE from inactive EoE.

Radiology

Several radiologic findings raise concern for the diagnosis of EoE including proximal esophageal narrowing, long segment narrowing, or prolonged retention of barium pill; however, there are no pathognomonic signs for EoE. Patients with a diagnosis of EoE may benefit from the use of contrast esophagram with barium pill to identify esophageal narrowing since occult strictures may be missed. In a retrospective study of 22 patients ranging in age from 2 to 20 years, 55% were found to have radiographic evidence of esophageal narrowing that had not been observed endoscopically [39]. Similarly, use of a barium-coated pill was shown to be of value in identifying occult narrowing in a retrospective study of three patients [40].

Histology

The histopathologic hallmark of EoE is dense esophageal eosinophilia with a diagnostic threshold of >15 eosinophils/HPF. The esophageal epithelium possesses characteristic features including thickening due to basal zone hyperplasia (normal is less than 3 cell layers), dilated intracellular spaces, and infiltration with other inflammatory cells including mast cells, T-cells, and B-cells (Table 9.3). Sampling of the lamina propria of the esophageal mucosa varies widely, but when obtained, it can appear fibrotic as defined by increased density of the collagen fibrils [41]. The EoE Histologic Scoring System (EoE-HSS) was developed to integrate elements of both eosinophilic and epithelial inflammation with grading (degree) and staging (extent) of each pattern [42]. Grade and stage scores are combined to create a composite score used to monitor therapeutic response.

Epidemiology

EoE incidence ranges from 2.1 to 12.8 cases per 100,000 people/year with pooled meta-analysis data reflecting a rate of 3.7/100,000 people/year (95% CI, 1.7–6.5) [43, 44]. Studies estimate EoE prevalence ranging from 2.3 to 90.7/100,000 people with pooled meta-analysis data reflecting prevalence of 22.7/100,000 (95% CI, 12.4–36) [43]. Prevalence is similar in Europe, North America, and Australia, but few studies have examined this in other countries. For instance, epidemiological data from other regions including Central/South America, Asia, and North Africa suggest that EoE is scarce [43].

A large retrospective study evaluated over 6,500 patients and found the male prevalence of EoE was twice that in females, and prevalence peaked in the 35–39-year age group with decreasing numbers after age 45 years [45]. Similar findings were reported with prevalence peaks during childhood and in the 30–40-year age group [9, 30].

Increased prevalence of EoE cannot be completely explained by increased recognition. Contributions from both genetic and environmental factors also play a role. For instance, several genetic variants include a single nucleotide polymorphism (SNP) in CCL26 which encodes eotaxin-3, downregulated epidermal barrier protein filaggrin, calpain 14 (CAPN14), and thymic stromal lymphopoietin (TSLP) [46]. Twin studies identified relative risk ratios for EoE within families ranging from 10 to 64 with higher values in male family members than female members [47]. EoE is passed on to 1.8–2.4% of relatives with 58% concordance among monozygotic twins and 36% concordance in dizygotic twins [47]. This higher-than-expected concordance among dizygotic twins further supports the role of environmental impact on EoE risk. Environmental factors that increase the risk of developing EoE include seasonality, lower population density, and early life events (prematurity, infection, antibiotic exposure) [47, 48]. In one study, breast feeding was associated with a decreased likelihood of developing EoE [47].

Table 9.3 Histologic findings associated with eosinophilic esophagitis

Peak eosinophil count >15/HPF
Epithelial basal zone hyperplasia: >15% of total epithelial thickness
Eosinophil abscesses: aggregate leading to epithelial architecture disruption
Eosinophil surface layering: rows of eosinophils in the upper one-third of epithelial layer
Dilated intracellular spaces with intercellular bridges
Lamina propria fibrosis/thickening
Surface epithelial alteration
Dyskeratotic epithelial cells

Collins et al. [128]

Clinical Associations with Esophageal Eosinophilia

At least four other clinical situations have been associated with the development of EoE or esophageal eosinophilia including *herpes simplex virus* (HSV) infection, oral or sublingual immunotherapy (OIT/SLIT), *Helicobacter pylori* infection, and celiac disease. Several case series document HSV infections preceding the development of EoE. For instance, in one study, 5 of 11 immunocompetent children with HSV esophagitis developed eosinophilic infiltration on follow-up esophageal biopsy [49]. Findings from this study and other case reports raise the question of whether HSV may be associated with EoE. An inverse relationship exists between *Helicobacter pylori* gastritis and the finding of esophageal eosinophilia [50]. This is curious given that *H. pylori* was definitively characterized in the 1980s, and the prevalence of infection has significantly decreased over a similar time course to the increased prevalence of EoE. Clinical observations have associated the use of SLIT or OIT with the frequent development of abdominal pain with subsequent endoscopic biopsies frequently demonstrating esophageal eosinophilia. To address this observation, 21 adults with IgE-mediated peanut allergy were evaluated with a baseline EGD prior to start of OIT [51]. The subjects had no GI symptoms but 5 were found to have >5 eosinophils/HPF and 3 had >15 eosinophils/HPF. Similarly, a meta-analysis in 2014 evaluating 711 patients demonstrated a rate of 3% that developed eosinophilia during immunotherapy course [52]. After discontinuation of OIT, symptoms and histology resolved in most cases. Whether this finding is a transient clinicopathologic finding or a chronic condition is yet to be determined.

Inherited connective tissue disorders have a predilection to develop various allergic phenotypes including EoE. Disorders such as Loeys-Dietz syndrome (LDS), Marfan syndrome, and Ehlers Danlos syndrome are caused by or associated with mutations in TGF-β. Implicated mutations result in increased protein levels of TGF-β which has been linked to the pathogenesis of EoE [53]. A retrospective study evaluated 58 patients with LDS (either heterozygous mutation TGF BR1 or TGF BR2) and found that 66% of patients reported GI complaints including poor growth, vomiting, abdominal pain, and dysphagia [54]. Ten of these patients had undergone upper endoscopy with 60% revealing histologic findings of EoE.

Esophageal eosinophilia can also be found in patients with celiac disease (CD). In a retrospective study, comparison of 421 patients with EoE and 763 patients with CD revealed that 3 children had both EoE and CD [55]. This corresponded with a 50- to 75-fold increased risk of each condition when diagnosed with the other. Treatment with gluten removal results in improvement in both conditions in 30–60% of patients [56].

Pathogenesis

EoE is a chronic inflammatory disease triggered by esophageal allergen exposure that leads to a cascade of events with increased Th2 cytokines, development of epithelial barrier dysfunction, and influx of inflammatory cells. Most often, this is triggered by food allergens, but recent evidence demonstrates the contribution by aeroallergens or other unknown stimuli [2, 14, 15]. In addition to basic studies identifying Th2 pathways, one of the strongest pieces of evidence supporting a food allergic cause is the remission induced by dietary elimination of proposed allergens [2].

Genetic Predisposition

A number of candidate genes increase susceptibility and subsequent development and progression of EoE. These include thymic stromal lymphopoietin (TSLP) on chromosome 5q22 and calpain 14 (CAPN14) on chromosome 2p23, as well as chromosome 1q21, the location of the epidermal differentiation complex which contains genes involved with squamous epithelial cell differentiation [2, 57]. One of the genes within this complex is filaggrin whose gene product is downregulated in EoE, thus leading to barrier dysfunction. Other contributing genes include STAT6 (Th2 adaptive immune response development and an intermediate for IL-4 and IL-13 signaling), EMSY (transcriptional regulation), and LRRC32 (a TGF-β binding protein) [2, 58, 59]. A study of 172 patient samples demonstrated a TSLP gain-of-function SNP found uniquely in EoE patient samples supporting this as a potential cause for increased disease risk compared to allergic controls [59]. This SNP was significantly increased in males with EoE compared to controls, but not in females suggesting one reason for increased EoE prevalence in males. Genome-wide association studies identified a dysregulated EoE transcriptome that is conserved in patients with EoE, but not found in patients with GERD [2, 6, 60]. The most highly expressed gene in the transcriptome is CCL26 which encodes eotaxin-3 with up to 53-fold increase compared to controls [6]. CCL26 is induced by IL-13 and is a critical mediator of eosinophilic inflammation. The importance of this gene in EoE pathophysiology is supported by an SNP in the CCL26 gene that portends increased disease susceptibility and in vivo murine models with eotaxin receptor (CCR3) deletions that provide protection from developing EoE [60]. Transcriptome analysis also revealed five mast cell genes that are highly induced in EoE supporting the role of this cell in disease pathogenesis [60]. Mast cells express the eotaxin receptor, CCR3, and thus their increased activity may be a result of elevated eotaxin-3 levels. Despite the discovery of multiple genetic susceptibility genes, the EoE disease concordance in dizygotic twins of 36% compared to nontwin siblings at 2.5% supports a complex interplay between genetic predisposition, environmental exposure, and epigenetics [47].

Inflammatory Cascade

Once esophageal epithelial cells are exposed to triggering antigens, TSLP transcription is upregulated via the Toll-like receptor 3 pathway (TLR3) leading to Th-2 adaptive immune response differentiation. Evidence for a Th-2 response in local dendritic cells is the increase in secretion of IL-4, IL-5, and IL-13 and upregulation of STAT6 downstream of the shared IL-4 and IL-13 receptor, IL-4Rα [2, 61]. IL-5 is a potent chemoattractant and activator of eosinophils; upregulation leads to eosinophil transmigration into the epithelium and increased eosinophil-derived granule protein deposition [2, 62]. Eosinophil granule products each have specific effects that may propagate EoE pathogenesis. Although not proven in EoE, eosinophil granule proteins are associated with a number of biological effects including the ability of major basic protein to disrupt epithelial barrier, eosinophil-derived cationic protein to increase cell membrane permeability, eosinophil-derived neurotoxin to potentiate the Th-2 response, and eosinophil peroxidase to induce local tissue injury [2, 46].

IL-13 also increases eosinophil chemotaxis and inflammation by two mechanisms. First, IL-13 can increase expression of eotaxin-3 via STAT6 leading to increased production of periostin, an independent facilitator of eosinophil

adhesion and recruitment [2, 46, 63]. Second, IL-13 can increase upregulation of CAPN14 that impairs esophageal epithelial barrier function by decreasing levels of the desmosome, desmoglein 1 (DSG1). CAPN14 also increases eosinophil chemotaxis and has direct effects on esophageal remodeling [58].

Barrier Dysregulation and Esophageal Remodeling

Esophageal inflammation and the EoE cytokine cascade can lead to development of impaired barrier function, likely due in part to downregulation of epidermal differentiation complex genes at 1q21 [64]. Impairment of the epithelial barrier occurs via IL-13 induction of CAPN14 which in turn decreases expression of desmosomal proteins DSG1 and filaggrin. DSG1 is one of the most downregulated genes in the mucosa of EoE patients, and diminished expression can result in increased intercellular spaces in the esophageal epithelium [57, 65]. Importantly, topical steroid treatment normalizes DSG1 protein levels [65]. Another implicated mediator of impaired barrier function is TGF-β. In vitro and ex vivo experiments have demonstrated that increased TGF-β decreases the epithelial tight junction molecule, claudin-7. Decreased claudin-7 allows for increased cell separation in the basal and suprabasal epithelial layers and resultant impaired barrier function [66]. Finally, eosinophil granule proteins can directly affect barrier function and raise the possibility of either initiating or perpetuating a response by allowing the passage of allergenic molecules [2, 65].

Esophageal remodeling is reflected by histologic changes including basal cell hyperplasia, dilated intracellular spaces, rete peg elongation, increased collagen deposition, fibrosis, and angiogenesis [2]. The etiology of underlying fibrosis in EoE is not certain, but a number of recent studies shed light on the potential role of TNF superfamily member 14 (LIGHT), elevated TGF-β through a functional SNP in the TGF-β gene, activation of TGF-β1 by Plasminogen activator inhibitor 1, and TGF-β-induced phospholamban expression [67–70]. These changes can lead to esophageal stiffening and/or dysmotility that increase the risk of dysphagia and food impactions [33, 71]. Two cell types, eosinophils and mast cells, are implicated in causing remodeling via increased levels of TGF-β. TGF-β potentiates esophageal remodeling via the SMAD signaling pathway (pSMAD2 and pSMAD3) [2, 63, 72]. This pathway upregulates fibrotic gene expression and leads to transformation of the resident fibroblast population to the contractile myofibroblast. Myofibroblasts can increase esophageal smooth muscle contraction with production of contractile proteins phospholamban and periostin. The EoE cascade may be cyclically potentiated by periostin that increases production of TSLP furthering the Th-2 pathway [65]. Successful treatment of EoE can decrease mast cells and TGF-β levels, thereby strengthening the hypothesis that mast cells are a large component in the pro-fibrotic changes and esophageal dysmotility observed in EoE [72].

Treatment

Current treatments for EoE include drugs, diet, and dilation [73]. A standardized definition for treatment response in EoE is lacking, but clinical trials currently use co-primary endpoints involving peak eosinophil counts and other histopathologic findings (see EoE-HSS) along with patient-reported symptoms. The overall goal of treatment includes improvement in symptoms, endoscopic and histologic findings, and normal growth and development [25].

Proton Pump Inhibitors

The AGREE consensus meeting of 2018 removed a PPI trial from diagnostic criteria for EoE and suggested that PPI-responsive esophageal eosinophilia (PPI-REE) is a likely subtype of EoE [10]. PPIs may have anti-inflammatory properties independent of acid blockade via blockade of the STAT6 transcription factor at the eotaxin-3 promotor [74–76]. This leads to the subsequent decrease of Th2 pathway eotaxin-3 secretion. Pre- and post-PPI treatment histopathology from 10 pediatric patients showed decreased expression of eotaxin-3 in the proximal esophagus and supported the hypothesis of acid-independent anti-inflammatory properties [74].

PPI therapy is effective for treating EoE demonstrated by a large meta-analysis of 618 patients (188 children) that showed clinical improvements in 61% of patients with histologic remission in 54% of children and in 50% of adults [77]. The reliability of PPI use to maintain remission continues to be studied. In a prospective study, 109 pediatric EoE patients were treated with high dose PPI therapy (1 mg/kg BID) for 8 weeks, and then responders were decreased to a maintenance regimen (1 mg/kg daily) for 12 months [78]. After the initial 8-week course, 66% of study participants were in histologic remission, and at follow-up endoscopy, a median of 14.5 months later, 70% of these patients remained in remission. Twelve of the subjects then had a dose decrease to 0.5 mg/kg daily for an additional 1 year with histologic remission maintained in 11 of 12.

One reason for variability in PPI responses may relate to PPI metabolism that can be divided into rapid, intermediate, and poor metabolizers. Phenotypes are dependent on CYP2C19 genotype, and the resultant plasma PPI levels and gastric pH levels are inversely proportional [79]. A retro-

spective cohort of 75 adult patients with initial PPI response were evaluated for long-term remission on PPI maintenance dosing in association with CYP2C19 genotyping [12]. Of the initial PPI responders, dosage was decreased to a lowest daily dose that maintained clinical remission with 45 patients receiving double dose (omeprazole 40 mg or equivalent) and 30 patients on single dose (omeprazole 20 mg or equivalent). Results of the follow-up endoscopies showed 55 patients with continued histologic remission and 20 that despite recurrence of esophageal eosinophilia remained asymptomatic. The only independent risk factors for patients with recurrence of esophageal eosinophilia were co-occurring rhinoconjunctivitis and patients with CYP2C19 rapid metabolizing genotype (leads to lower plasma PPI levels) showing an odds ratio (OR) of 12.5 and 8.6, respectively.

Adverse drug reactions associated with PPI use are rare, but the most common effects include nausea, abdominal pain, flatulence, headache, and diarrhea [80]. Additional adverse effects associated with longer term use have been described, both related and unrelated to acid inhibition [81].

Steroids

Systemic and topical corticosteroids induce clinical and pathological remission in EoE patients [82]. In 2008, a randomized clinical trial (RCT) compared systemic steroid and topical steroid therapies in 80 patients with EoE, and both had clinical and histologic improvement within 4 weeks, with minimal differences in efficacy [83]. Placebo-controlled trials using topical steroids including fluticasone propionate (FP) and oral viscous budesonide (OVB) have shown a 66% histopathologic improvement [84]. FP is dosed via a metered dose inhaler (MDI) inhaler, but instead of inhaling, the medication is puffed into the mouth and swallowed [85, 86]. OVB uses liquid budesonide mixed with Splenda or another thickening agent to increase viscosity for amplified contact time in coating the esophagus [87]. In a randomized study of 24 children, OVB induced a significant clinicopathological response compared to those treated with placebo [88]. A study comparing the efficacy of OVB and FP showed no significant difference in histologic remission at 71% and 64%, respectively [86]. Another topical steroid formulation used for EoE, ciclesonide, is delivered as a pro-drug that is converted to the active form des-CIC by esterases on the esophageal epithelial surface [89]. In one pilot study, ciclesonide led to clinicopathological remission, and in another, findings were less clear; further study is required to see if ciclesonide will offer continued benefit [90, 91].

All steroid medications raise concerns for systemic absorption and complications such as adrenal insufficiency, altered bone metabolism, and slowed linear growth, but these are uncommon with topical formulations. Comparison of systemic steroids with topical formulations showed 40% of the patients receiving systemic steroids developed weight gain, cushingoid features, and hyperphagia compared to none in the topical group [83]. The most common adverse drug reaction seen with topical steroid formulations is esophageal *Candida* infection with between 12% and 16% of patients effected [83, 86]. The rate of adrenal insufficiency in patients treated with topical corticosteroids varies between studies from 5% to 66% with the largest studies showing that adrenal insufficiency is unusual, and when it occurs, patients are generally also taking other topical steroid formulations for concomitant atopic conditions [92–96].

Elimination Diets

In the first therapeutic study using dietary elimination, an elemental diet induced a clinical and histological response and allergenic food addition led to disease reactivation [5]. This study, as well as others, showed the benefits of food elimination, but because of impact on quality of life and other factors, this may not be suitable for all [97, 98]. No testing platform is available to identify EoE-related food allergens reliably and thus an empiric approach remains the standard of care [98, 99].

Implementation of an empiric six-food elimination diet (SFED; excludes milk, soy, egg, wheat, peanuts/tree nuts, fish/shellfish) leads to a clinicopathological response rate of 72% [84]. This diet was first evaluated in a retrospective study from 2006 of 60 children receiving either SFED or elemental formula diet [98, 99]. Results of this study showed 74% histologic remission in SFED compared to 88% in the elemental formula diet. Wheat and milk have been found to be leading EoE causative antigens at 58% and 68%, respectively, but with significant heterogeneity between studies [98]. After empiric elimination, foods were added back every 6 weeks with repeat endoscopy in order to determine specific food triggers [99, 100].

Four food elimination diet (FFED; excludes milk, soy, egg, and wheat) is another dietary intervention with 64% achieving histologic remission, symptom remission in 34%, and symptom improvement in 91% [100]. Since milk is thought to be the most frequently implicated trigger in rechallenged patients, milk-only elimination has been compared to the FFED in a recent RCT [101]. Results showed equivalent histologic improvement, slightly lower symptomatic improvement in the milk-only group, but a significant improvement in the quality-of-life scores of milk-only elimination.

Recently, a step-up approach was proposed starting with the two most likely trigger foods, milk (all dairy) and gluten (stricter than just wheat to avoid cross-reactivity) [102]. This two-food elimination diet (TFED) led to histologic response

in 43% of participants. Those that did not respond were stepped up to the FFED. The step-up process helped limit the number of endoscopies required to find a food trigger by 20% during reintroduction. Limitations of elimination diets include potential reduction in quality of life and need for repeat endoscopic evaluation with the attendant costs and potential complications.

Allergy Medications

Medications used in other allergic conditions such as cromolyn, montelukast, and omalizumab are not effective in treating EoE. Lack of effect is likely because EoE is not an IgE-mediated allergic condition. In a retrospective study of 381 pediatric EoE patients, 14 were treated with cromolyn, a mast cell stabilizer, with no change in peak eosinophil count or symptom improvement [103]. Similarly, in an RCT of 41 adults that investigated maintenance of topical steroid induced remission, no symptomatic or histological differences were observed between montelukast, a leukotriene antagonist, and placebo [104]. The anti-IgE monoclonal antibody, omalizumab, has also been used in a small RCT of 30 adults and no difference in symptoms or eosinophil counts was reported [105].

Biologic Treatments

A number of clinical trials are studying novel biologic therapies that target various points in the EoE cytokine cascade.

Medications targeting IL-5 include IL-5 monoclonal antibodies mepolizumab and reslizumab and the IL-5Rα blocker benralizumab. Anti-IL-5 trials in both adults and pediatrics have shown reductions in mean eosinophil counts [106, 107]. However, in the adult study, no histologic remission (<15 eosinophils/HPF) was seen and only 8.8% of patients in the pediatric trial met the primary endpoint of <5 eosinophils/HPF with no difference in symptom improvement compared to placebo. A phase II RCT tested benralizumab in 20 patients with hypereosinophilic syndrome. Results showed both clinical and histologic efficacy as well as safety with no adverse events that limited treatment [108].

Several trials have studied both direct IL-13 blockade and IL-13 downregulation via IL-4Rα, the shared signaling intermediate between IL-13 and IL-4. The most promising has been the IL-4Rα blocker, dupilimab. In a phase II, placebo-controlled RCT with 47 patients, results showed significant improvement in symptoms, endoscopic and histologic findings, and a decrease in peak eosinophil count with 82.6% of patients below the threshold of <15 eosinophils/HPF compared to none in the placebo group [109]. Other trials have studied IL-13 monoclonal antibodies RPC4046 and QAX575 both of which have shown significant decrease in mean eosinophil counts compared to placebo. The RPC4046 study exhibited significant improvements in symptoms, EREFS score, and histology (EoE-HSS) with the most pronounced improvement found in steroid refractory patients [110]. The QAX575 study did not meet primary endpoint of 75% reduction in peak eosinophil count, and only demonstrated a trend toward decreased symptoms [111].

Other Medications

Multiple additional targets are under investigation including sialic acid binding Ig-like lectin 8 (Siglec-8), TSLP, integrins, and TGF-β. Siglec-8 is exclusively expressed by eosinophils and mast cells, and the Siglec-8 antibody (AK002) has preliminary data showing selective depletion of eosinophils and mast cells in blood and tissue [112]. An antibody directed toward TSLP, tezepelumab (AMG 157), is currently used in allergic asthma therapy but has not been studied in EoE [113]. Vedolizumab binds to integrin α4β7 and can decrease eosinophil recruitment in vitro. In a study of 5 patients with difficult-to-treat eosinophilic gastroenteritis, vedolizumab treatment led to a clinical and histologic improvement [114]. Finally, inhibition of TGF-β with the antihypertensive drug Losartan is currently under investigation [115].

Dilation

Esophageal dilation with either Maloney dilator (bougie) or through-the-scope (TTS) balloon dilation is an important component of EoE therapy that can improve dysphagia and reduce food impactions. Dilation does not reduce inflammation and should be combined with medical treatment or elimination diets to facilitate symptom improvement as well as histological remission [116].

Goals of treatment are to increase esophageal diameter to permit passage of food stuffs and limit food impactions. The desired outcome of dilation is symptomatic improvement of dysphagia and/or a mucosal tear or rent. The later finding indicates a stopping point and subsequent repeated dilations may be necessary [116, 117]. In adults, up to 95% of patients have improvement in symptoms after esophageal dilation with median duration of effect of 12 months [117]. Pediatric dilation studies are limited; however, a retrospective study evaluated 40 patients between 4 and 8 years of age with EoE who underwent 68 dilations [118]. The goal of initial dilation was 15 mm or the development of a deep rent. Study results showed an increase in pain, fever, or symptoms requiring evaluation in a clinic or emergency department compared to endoscopy without dilation, however no difference in adverse

event rate in dilated EoE patients compared to patients dilated for non-EoE indications.

The most common adverse event of dilation is pain. Perforation is unusual when dilations are performed sequentially [118]. In a 2017 meta-analysis of 845 EoE patients (87 pediatric patients), chest pain was the most common adverse event associated with dilation seen in up to 17% of cases [117]. Perforation in this study was rare occurring in 0.033% of procedures with only 1 reported transmural perforation. A prospective study of 105 patients who underwent 246 dilation procedures showed no difference in adverse events whether the dilation was performed with a bougie or a TTS balloon [119]. The only factor that significantly increased risk of adverse events was age with an increase of 12% for each additional year of life. When performed conservatively, dilation is a safe and effective component to EoE therapy, and the method of dilation should be guided by endoscopist experience and by type of stricture or narrowing present.

Maintenance Therapy

EoE is a chronic inflammatory illness which recurs after cessation of therapy [103]. Natural history studies show that untreated disease may progress to a fibrostenotic phenotype with esophageal narrowing and stricture [4, 19]. Because of the poor correlation between symptoms with endoscopic and histologic findings, and due to lack of clear consensus treatment goals (symptom improvement, endoscopic improvement, histologic remission, quality of life, etc.), expert opinion supports the use of a long-term anti-inflammatory regimen. Several studies have explored decreased dosing with surveillance of ongoing remission. One study found that decreasing PPI from BID to once daily maintained remission in 75–85% of patients [120]. Results are mixed on maintenance of remission with long-term topical steroids with one study showing that a decrease in daily fluticasone dosing from 1760 mcg to 880 mcg maintained remission in 73% of patients [85]. However, recent data from a retrospective study of 82 adult patients followed for a median of 2.2 years revealed a histologic relapse rate of 67% with no difference between high dose and low dose regimen (>0.5 mg or <0.5 mg daily dosing of budesonide respectively). In this study, high dose therapy did lead to longer time prior to histologic relapse [121]. Even less data are available to guide maintenance strategy for dietary elimination; however, studies consistently demonstrate continued remission in those that maintain elimination of trigger foods [122]. Recent data suggest that initially swallowed steroids are the most cost-effective choice, but long-term, elimination diets with determination of the food trigger may be the cheaper option [123].

Disease Monitoring

Once diagnosed with EoE, monitoring for resolution and/or recurrence of esophageal inflammation is important to detect ongoing inflammation that could lead to esophageal remodeling and fibrosis [33, 44]. Peripheral eosinophilia does not correlate accurately with esophageal eosinophil count or ongoing inflammation [124]. Monitoring may also be indicated with new symptom development or during re-exposure to eliminated food groups with dietary therapy. Sedated endoscopy is required for disease surveillance to obtain biopsies for assessment of disease. In some studies, endoscopy is performed 6 weeks after food reintroduction [102]. Outside of dietary therapy, no consensus guidelines exist on frequency of endoscopy.

Alternatives to sedated endoscopy include transnasal endoscopy (TNE) and others for monitoring EoE-related inflammation. A retrospective study published in 2019 of 294 TNEs completed in pediatric patients age 3–22 years demonstrated a 98% success rate with reduced cost and with no adverse events [125]. Biopsies obtained via this method had no difference in surface area of epithelial samples compared to previous EGD, and lamina propria was present in 35–40% (depending on size of forceps) compared to 49% with EGD samples. This study supports TNE as an alternative for certain patients. Other methods currently under investigation include the esophageal string test and the cytosponge. A recent prospective trial evaluated the esophageal string test in 134 participants with a 1-hour dwell time [124]. Results showed that eosinophil associated proteins (EAP) extracted from an esophageal string displayed strong correlation with EAP from endoscopically obtained biopsies and accurately identified active EoE, inactive EoE, and normal esophagus. Similarly, a prospective study of 80 adult patients who underwent 105 cytosponge procedures showed strong correlation with EGD biopsy ($r = 0.78$) and a sensitivity and specificity of 75% and 86%, respectively, in identifying inactive disease (<15 eosinophils/HPF) [126]. Recently, a novel EoE oral imaging agent, ^{99m}Technetium-labeled heparin, has been described that binds to major basic protein and can non-invasively evaluate and monitor EoE inflammation with single-photon emission CT (SPECT) [127]. A study involving five adult patients showed an ability to differentiate between patients with active eosinophilic inflammation, inactive EoE, or GERD with no adverse events.

Summary

Since the initial characterization in the 1990s of EoE as a discrete entity, great strides have been made in understanding the pathogenesis, diagnosis, and effective therapeutic

interventions. Endoscopic and histologic scoring systems, EREFS and EoE-HSS, now allow for more reliable measurement of disease activity and more consistent standardization for continuing natural history and treatment studies. The increasing incidence of EoE and the morbidity associated with untreated inflammation has led to a rapid push toward the development of disease-specific therapies both in the category of topical corticosteroids and more directed medications like monoclonal antibodies. Additionally, investigation is ongoing for less invasive testing and noninvasive biomarkers for disease surveillance. With the breakthroughs that have been made in the last 10 years and the current research in progress, it is likely that the diagnosis, treatment, and monitoring of EoE will continue to change rapidly in the near future.

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Gastroesophageal Reflux

10

Yvan Vandenplas and Sébastien Kindt

Abbreviations

AAF	Amino acid formula
AR	Antireflux
BAL	Broncho-alveolar liquid
CM	Cow's milk
CMPA	Cow's milk protein allergy
eHF	Extensively hydrolyzed formula
ENT	Ear-nose-throat
GER(D)	Gastroesophageal reflux (disease)
H2 RA	H2 receptor antagonist
LES	Lower esophageal sphincter
LLM	Lipid-laden macrophages
NERD	Nonerosive reflux disease
PPI	Proton-pump inhibitor
TLESR	Transient lower esophageal sphincter relaxation

Introduction

The European and North American Societies of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN/NASPGHAN) published exhaustive consensus documents on the definition, diagnosis and management in 2009, which were updated in 2018 [1, 2]. There is a trend to diagnose gastroesophageal reflux disease (GERD) more frequently. Between 2000 and 2005, the diagnosis of GERD and acid-related conditions among infants more than tripled (from 3.4% to 12.3%) and increased by 30–50% in other age groups [3]. Whether this means a more appropriate diagnosis or a trend toward overdiagnosis is debatable.

Labeling an otherwise healthy infant as having a "disease" increases the parental interest in medicating their infant even when parents were told that medications are not effective [4]. These findings suggest that use of disease labels may promote overtreatment by causing people to believe that ineffective medications are both useful and necessary [4].

Definitions

Gastroesophageal reflux (GER) is the involuntary passage of gastric contents into the esophagus. GER is a normal physiological process occurring several times per day in every human, particularly after meals [5]. Most reflux episodes are of short duration, asymptomatic, and limited to the distal esophagus. Typically, a reflux episode is the consequence of a transient lower esophageal sphincter relaxation (TLESR), unrelated to prior swallowing. Physiologic GER is the consequence of increased abdominal pressure not accompanied by an increase of the LES pressure or when GER is associated with absence of symptoms, or during the first months of life accompanied with regurgitation, and occasionally with vomiting [2]. Healthy and sick individuals do not differ in the presence or absence of GER, but in the frequency, duration, and intensity of GER and in the association with symptoms or complications. Physiologic reflux becomes pathologic if esophageal clearance is insufficient, if acid buffering is insufficient, if gastric emptying is delayed, if abnormalities in epithelial restitution or repair occur, if there are anatomical abnormalities such as hiatal hernia, etc. Both in children and adults, GERD is defined as reflux causing troublesome symptoms and/or complications [2, 6]. GERD is reflux associated with esophageal and/or extra-esophageal symptoms impairing quality of life and/or mucosal damage. The vast majority of patients with GERD show no abnormalities on endoscopy or on histology from esophageal biopsies and suf-

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fer nonerosive reflux disease (NERD). To be defined as GERD, reflux symptoms should be troublesome to the infant, child, or adolescent and not limited to the caregiver [2]. A major issue is the estimation of troublesome symptoms in young children because they cannot adequately report symptoms [5, 7]

Regurgitation, spitting up, posseting, and spilling are synonyms and are defined as the passage of refluxed gastric contents into the pharynx and above [2]. Regurgitation is mainly effortless, but may sometimes also be forceful. Even in infants, only a minority of the reflux episodes are accompanied by regurgitation. Regurgitation is a characteristic symptom of reflux in infants, but is neither necessary nor sufficient for a diagnosis of GERD, because regurgitation is not a sensitive nor a specific criterion [2, 5]. Up to 50% of all infants under the age of 4 months present at least one to several episodes of spilling per day [2, 8]. Regurgitation is distinguished from vomiting by the absence of a central nervous system emetic reflex, retrograde upper intestinal contractions, nausea, and retching. Vomiting is defined as an expulsion with force of the refluxed gastric contents from the mouth [2, 5]. Vomiting is a coordinated autonomic and voluntary motor response, causing forceful expulsion of gastric contents [2]. Vomiting associated with reflux is likely the result of the stimulation of pharyngeal sensory afferents by refluxed gastric contents. Bilious vomiting should not be diagnosed as GERD. Otherwise, healthy infants and children with reflux symptoms that are not troublesome and are without complications should not be diagnosed with GERD [1, 2]. In adults, heartburn or pyrosis is the most typical manifestation of GERD [6]. However, some adult patients complaining of heartburn do not have GERD, they have a syndrome called functional heartburn [9]. Since heartburn causes distress and pain, crying and distress in infants are often considered as the manifestation of heartburn in infants. However, there is no evidence to sustain this hypothesis [2, 10].

"Rumination" is characterized by a voluntary contraction of the abdominal muscles resulting in the habitual regurgitation of recently ingested food that is subsequently spitted up or reswallowed. Gagging, mouthing, and swallowing of refluxed material is identified as rumination [11].

Prevalence, Environmental and Genetic Factors

Determination of the exact prevalence of GER and GERD at any age is virtually impossible for many reasons: most reflux episodes are asymptomatic; symptoms and signs are nonspecific; and self-treatment is common. Alcohol, smoking, drugs, food components, excessive solid food and liquid intake, and overweight are GER-inducing variables. In adults, over-the-counter use of many medications such as aspirin and nonsteroidal anti-inflammatory drugs favor GER [12]. Race, sex, body mass index, and age are independently associated with hiatus hernia and esophagitis [13]. In adults, the prevalence of GERD (defined as symptoms of acid regurgitation, heartburn, or both, at least once a week) varies widely by country, with typical figures between 10% and 20%, whereas in Africa the prevalence was estimated at 7.6% [14]. In Asia, the prevalence ranges from less than 10% in China to more than 25% in India [6, 15–17].

About 20% of infants regurgitate more than 4 times a day and about 20% of mothers consider regurgitation as troublesome [2, 8]. Epidemiological data suggest that about 25% of adults sought medical advice for GER-related symptoms during the past year, with higher values with increasing age [18–21]. French data report a prevalence of GER in 10% and of GERD in 6% of all children 0–17 years old [19]. According to data obtained by the Health Improvement Network Incidence, the incidence of GERD in children is 0.84/1000 person-years [18]. The incidence decreases with age from 1.48/1000 person-years among 1-year-old children until the age of 12 years, whereupon it again increases to a maximum at 16–17 years of 2.26/1000 person-years for girls and 1.75/1000 person-years for boys [18]. According to epidemiologic data, children with GERD had double the risk to suffer an extra-esophageal condition such as asthma, pneumonia, or cough compared with children and adolescents with no diagnosis of GERD [18].

The rapidly increasing prevalence of obesity is related to the rising prevalence of GERD [6]. Total and abdominal obesity are risk factors for the development of GERD symptoms in children [22].

An autosomal dominant inheritance of hiatal hernia was described by discovering familial hiatal hernia in five generations of a large family, but without demonstrating the link to GERD. The genetic influence on GERD is supported by increased GER symptoms in relatives of GERD patients. GERD is associated with GNB3 C825T [23]. The concordance for GER is higher in monozygotic than dizygotic twins [24]. Family studies have revealed about 31% heritability of the disease [25]. Genes in question have been localized to chromosomes 9 and 13. A locus on chromosome 13q, between microsatellite D13S171 and D13S263, has been linked with severe GERD in five multiple affected families [26]. This could not be confirmed in another five families, probably due to genetic heterogeneity of GERD and different clinical presentation of patients [27]. A large GERD GWAS meta-analysis (80,265 cases, 305,011 controls) identified 25 independent genome-wide significant loci for GERD [28]. Further, 91% of the GERD risk-increasing alleles also increase Barrett's esophagus and esophageal adenocarcinoma, greatly expanding gene discovery for these traits [28]. Despite GERD polygenic basis, specific genetic loci such as rs10419226 on chromosome 19, rs2687201 on

chromosome 3, rs10852151 on chromosome 15, and rs520525 on the paired related homeobox 1 gene have been mentioned as potential risk factors [25]. The relevance of these findings for the general population remains unclear.

Pathophysiology

Transient lower esophageal sphincter relaxations (TLESRs) are the most important pathophysiologic mechanism causing GER at any age, from prematurity into adulthood [2, 6, 29]. TLESRs are a neural reflex, triggered mainly by the distention of the proximal stomach and organized in the brain stem, with efferent and afferent pathways traveling in the vagal nerve, activating an intramural inhibitory neuron which releases nitric oxide to relax the LES.

GER is influenced by genetic, environmental (e.g., diet smoking), anatomic, hormonal, and neurogenic factors (Fig. 10.1) [29]. Three major tiers of defense serve to limit the degree of GER, and to minimize the risk of reflux-induced injury to the esophagus. The first line of defense is the "antireflux barrier," consisting of the LES and the diaphragmatic pinchcock and angle of His. Compared to adults, infants have a short intraabdominal esophagus. When this line of defense fails, the second line of defense, esophageal clearance, assumes greater importance and limits the duration of contact between luminal contents and the esophageal epithelium. Anticipating the section on diagnostic techniques: results of esophageal pH monitoring and impedance taught us that chemical clearance is much slower than bolus or volume clearance (whether that is true or due to small remnants of refluxed acid that stick longer to the pH measuring material remains open for debate). Gravity and esophageal peristalsis serve to remove volume from the esophageal lumen, while salivary and esophageal secretions from esophageal submucosal glands neutralize acid. The third line of defense, tissue or esophageal mucosal resistance, is relevant when acid contact time is prolonged [29]. Esophageal mucosal defense can be divided in pre-epithelial (protective factors in saliva and esophageal secretions containing bicarbonate, mucin, prostaglandin E2, epidermal growth factor, transforming growth factor), epithelial (tight junctions, intercellular glycoprotein material), and post-epithelial factors [29]. There is a very important interindividual variation of reflux perception suggesting different esophageal sensitive thresholds. Additionally, the sensitivity threshold varies along the esophagus, with reflux reaching the proximal esophagus being more frequently perceived [30].

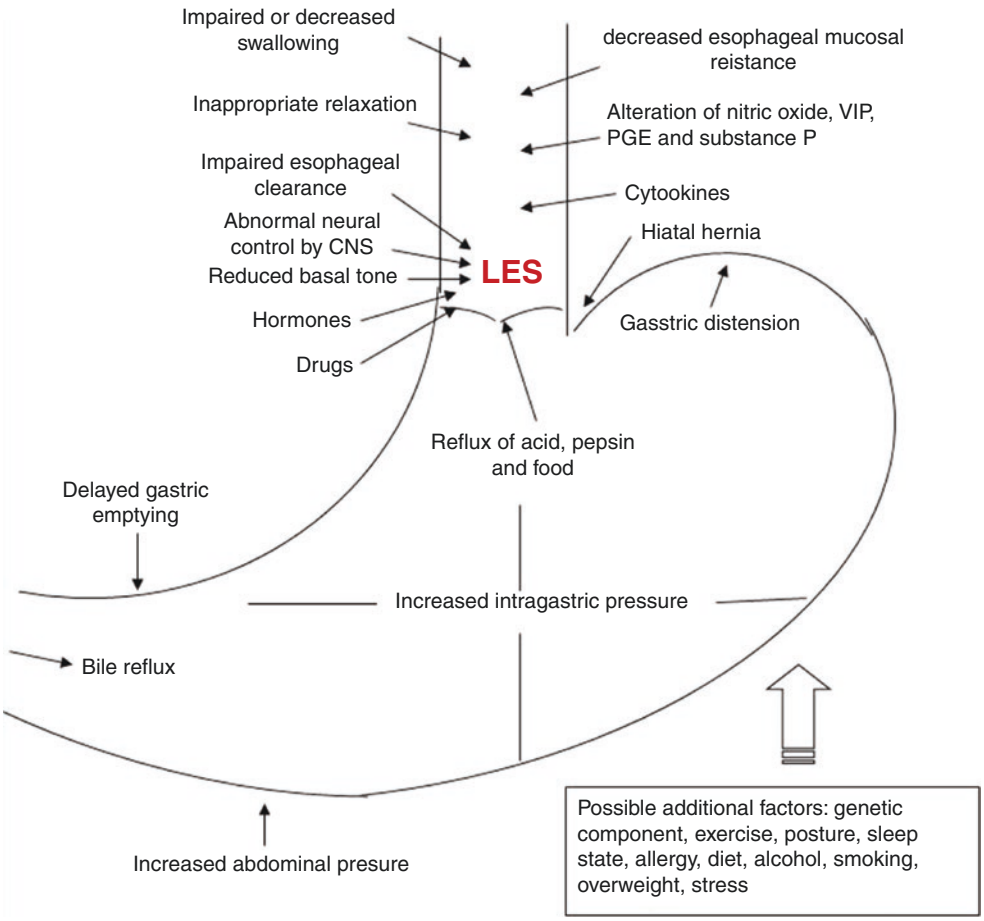


Fig. 10.1 Pathophysiologic mechanisms for GER. (Ref. [149])

Capsaicin levels and the transient receptor potential vanilloid receptor-1 play a role in the sensation of heartburn [31]. The esophageal mucosa contains acid-, temperature-, and volume-sensitive receptors. Proximal and distal esophageal mucosa of adult patients with nonerosive reflux disease has more superficial afferent nerves compared with controls or patients with erosive reflux disease or Barrett's esophagus [32]. Acid hypersensitivity in patients with nonerosive reflux disease might be partially explained by the increased proximity of their afferent nerves to the esophageal lumen, and therefore greater exposure to noxious substances in refluxate [32]. However, in infants, nonacid reflux is reported to cause more frequently and more severe crying and stress in infants than acid reflux [33, 34]. A widening of the intercellular spaces is reported in patients with esophagitis and in patients with endoscopy-negative disease [35]. Esophageal sensitivity to acid decreases when the esophagitis has healed. The presence of fat in the duodenum increases the sensitivity to reflux. Hyposensitivity as occurs in patients with Barrett's esophagus is a secondary phenomenon.

GER occurs during episodes of TLESR or inadequate adaptation of the sphincter tone to changes in abdominal pressure. All the factors responsible for maintaining the LES tone are not yet determined, but nitric oxide likely plays an important role [36].

Infants ingest more than twice the volume than adults per kg bodyweight (100–150 ml/kg/day compared to 30–50 ml/kg/day), causing more gastric distention, and as a consequence more TLESRs. Feeding frequency is higher in infants than in adults, resulting in more postprandial periods during which TLESRs are more common. When investigated in supine position, the frequency of TLESRs in healthy adults and these with acid GERD does not differ. In healthy adults, only 30% of the TLSERs are accompanied by acid reflux, but in patients with GERD, reflux occurs in 65% of the TLESRs. Thus, in adults, controls and GERD patients have the same number of TLESRs, but in patients with GERD, these TLESRs are more than twice as frequently accompanied with acid GER [37]. Normal individuals rarely experience TLESRs during sleep. Supine position eliminates all the beneficial gravitational effects of the upright position. Noxious materials, rather than air, are positioned at the cardia, available to move into the esophagus during TLESRs. A reflux is more likely to reach the pharynx in the recumbent than in the upright position. Both salivation and swallowing are markedly reduced during sleep, further impairing clearance. The upper esophageal sphincter is atonic during sleep, allowing reflux almost free to access the airways.

Delayed gastric emptying may increase postprandial reflux possibly by increasing the rate of TLESRs. Delayed gastric emptying has been documented in infants and children with symptomatic GER, particularly those with neurologic disorders. Abnormal gastric accommodation to a meal and prolonged postprandial fundic relaxation has been described in patients with GERD [38]. Esophageal acid exposure in patients with GERD is directly correlated with the emptying time of the proximal stomach. GERD was classically considered to be an acid peptic disease. But as a group, the majority of patients with reflux disease do not have a significant increase in gastric acid secretion. Recent analysis of postprandial acidity in the area of the gastroesophageal junction suggests that local acid distribution ("the gastric acid pocket") rather than total gastric secretion might be more relevant to the pathogenesis of GERD. The importance of the gastric acid pocket has been well established in adults, but has not been studied in children. Differences may exist in the degree of mixing of fundic contents leading to different distributions of acid in the stomach. Studies using pH monitoring, scintigraphy, and gastric magnetic resonance suggest that gastric mixing can be incomplete. Different layers of viscosity within the stomach might therefore influence the distribution of the gastric contents. A collection of acid in the gastric part of the esophageal junction was shown in adults in supine position, even in the postprandial period when stomach content was neutralized by the meal [38].

Hiatal hernia increases the number of reflux episodes and delays esophageal clearance by promoting retrograde flow across the esophagogastric junction when the LES relaxes after a swallow. This mechanism underlies the so-called rereflux phenomenon (recurrence of acid reflux while the esophageal pH is still below 4 after a previous acid reflux event) [39].

The majority of the studies on pathophysiologic mechanisms have been performed in adults and did not consider weakly acid and nonacid reflux. The refluxed material can be acid, weakly acid, or nonacid. Reflux may be a mix of gas and liquid or pure liquid, and may or may not contain bile. More than half of the acid and weakly acid reflux episodes are associated with reflux of gas [37]. Weakly acid reflux also occurs predominantly during TLESRs. With liquid meals, patients with GERD had a similar total rate of reflux episodes but a higher proportion of acid reflux events than controls [40]. Weakly acid reflux may be responsible for remaining symptoms in patients under antisecretory treatment. Components contributing to the noxiousness of refluxate are pepsin, bile acids and salts, and trypsin. The latter two depend on duodenogastric reflux preceding GER and are implicated in the genesis of strictures and Barrett's esophagus. Acid is emptied from the esophagus with one or two sequences of primary peristalsis, then the residual acidity in neutralized by swallowed saliva. Secondary peristalsis is the response to esophageal distension with air or water and is more important during sleep when peristalsis is reduced. Patients may have normal primary peristalsis but abnormal secondary peristalsis. Thus, nonacid reflux as occurs in the

postprandial period may be inefficiently cleared and cause prolonged esophageal distension, and thus cause symptoms of discomfort [33]. Esophageal clearance modulates the duration of reflux episodes, while mucosal resistance modulates the noxiousness of the components of refluxate. GER causes respiratory symptoms through different pathways such as (micro-)aspiration or may be vagally mediated (Fig. 10.2).

Neither *H. pylori* infection nor eradication of *H. pylori* obviously causes GERD [2, 41]. The presence of *H. pylori* is not an important pathophysiological factor in gastroesophageal reflux. On the other hand, its relationship with esophagitis appears to be inverse ratio. The fact that the *H. pylori* presence is statistically greater in the grade A esophagitis could confirm the hypothesis that the bacteria would slow down the development of the esophagitis [42]. Improvement in epigastric pain is significantly correlated with the improvement in GER symptoms but not with eradication of *H. pylori* [41].

The role of the upper esophageal sphincter in GERD and chronic respiratory disease, laryngitis, hoarseness, coughing, etc. has been insufficiently studied. Only patients with supraesophageal reflux disease were reported to have abnormal upper esophageal sphincter relaxation responses to rapid distension with saline [43]. The upper esophageal sphincter relaxes in response to esophageal body distention by gas, in contrast to its contractile response to esophageal body distension by fluid. Symptom presentation has not been linked to different pathophysiological mechanisms. Children presenting with upper airway disease or ear-nose-throat (ENT) manifestations may rather suffer from an insufficient upper esophageal sphincter, while patients with esophagitis may have more noxious reflux, insufficient clearing mechanisms, or a poor esophageal mucosal resistance. However, this has not been studied and is thus not demonstrated.

Symptoms and Signs

In normal 3- to 4-month-old infants, 3 to 4 episodes of GER are detectable during 5 min of intermittent fluoroscopic evaluation [44]. Normal ranges of esophageal pH monitoring report up to 31 ± 21 acid reflux episodes recorded within a 24-h period, but sample frequency, data handling by the recording device, and program do determine this incidence [45]. According to normal ranges proposed for impedance, a normal range up to 100 reflux episodes in 24 h has been proposed [46]. However, personal experience suggests that 100 reflux episodes is likely to be too high to be considered as normal. Normal acid clearance time was reported to be 151 s, while normal bolus clearance time was 25 s [47]. Less than 10% of infants and children have (acid and troublesome) GERD [45]. However, for ethical reasons, investigations were performed in symptomatic children.

While reflux does occur physiologically at all ages, there is also a continuum between physiologic GER and GERD (Tables 10.1 and 10.2). GERD is a spectrum of a disease that can best be defined as manifestations of esophageal or adjacent organ injury secondary to the reflux of gastric contents into the esophagus or, beyond, into the oral cavity or airways. The symptoms indicating the possibility of GERD differ according to age (Table 10.1). The list of most frequent differential diagnoses of vomiting in infants and children is listed in Table 10.3. Most important is to recognize alarm

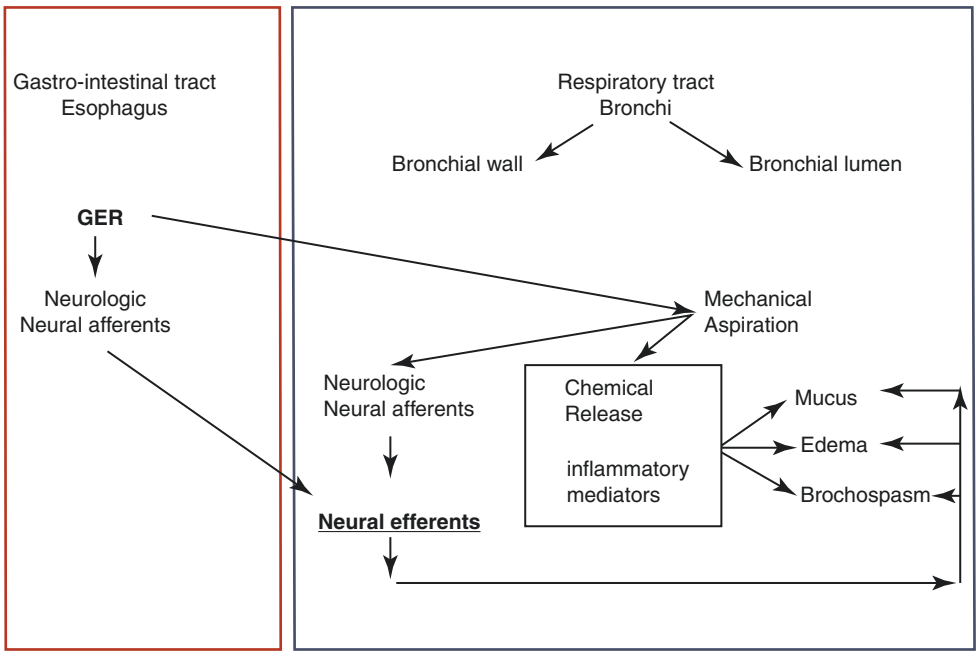


Fig. 10.2 Pathophysiologic mechanisms of GER causing respiratory disease

Table 10.1 Symptoms and signs that may be attributed to GER(D) according to age

Manifestations	Infants	Children	Adults
Impaired quality of life	+++	+++	+++
Regurgitation	++++	+	+
Excessive crying/irritability	+++	+	–
Vomiting	++	++	+
Food refusal/feeding disturbances/ anorexia	++	+	+
Persisting hiccups	++	+	+
Failure to thrive	++	+	–
Abnormal posturing/Sandifer's syndrome	++	+	–
Esophagitis	+	++	+++
Persistent cough/aspiration pneumonia	+	++	+
Wheezing/laryngitis/ear problems	+	++	+
Laryngomalacia/stridor/croup	+	++	–
Sleeping disturbances	+	+	+
Anemia/melena/hematemesis	+	+	+
Apnea/ALTE/desaturation	+	–	–
Bradycardia	+	?	?
Heartburn/pyrosis	?	++	+++
Epigastric pain	?	+	++
Chest pain	?	+	++
Dysphagia	?	+	++
Dental erosions/water brush	?	+	+
Hoarseness/globus pharyngeus	?	+	+
Chronic asthma/sinusitis	–	++	+
Laryngostenosis/vocal nodules problems	–	+	+
Stenosis	–	(+)	+
Barrett's/esophageal adenocarcinoma	–	(+)	+

Legend: +++ very common; ++ common; + possible; (+) rare; – absent; ? unknown

Table 10.2 Warning signals requiring investigation in infants with regurgitation or vomiting

Bilious vomiting
GI bleeding
Hematemesis
Hematochezia
Consistently forceful vomiting
Onset of vomiting after 6 months of life
Failure to thrive
Diarrhea
Constipation
Fever
Lethargy
Hepatosplenomegaly
Bulging fontanelle
Macro/microcephaly
Seizures
Abdominal tenderness or distension
Documented or suspected genetic/metabolic syndrome

Table 10.3 Differential diagnosis of vomiting in infants and children (adapted from Refs. [1, 2])

GI obstruction	Pyloric stenosis
	Malrotation with intermittent volvulus
	Intestinal duplication
	Hirschsprung disease
	Antral/duodenal web
	Foreign body
	Incarcerated hernia
Other GI disorders	Achalasia
	Gastroparesis
	Peptic ulcer
	Eosinophilic esophagitis/gastroenteritis
	Food allergy
	Inflammatory bowel disease
	Pancreatitis
	Appendicitis
Neurologic	Hydrocephaly
	Subdural hematoma
	Intracranial hemorrhage
	Intracranial mass
	Infant migraine
	Chiari malformation
Infectious	Gastroenteritis
	Sepsis
	Meningitis
	Urinary tract infection
	Pneumonia
	Otitis media
	Hepatitis
Metabolic/ endocrine	Galactosemia
	Hereditary fructose intolerance
	Urea cycle defects
	Amino and organic acidemias
	Congenital adrenal hyperplasia
Renal	Obstructive uropathy
	Renal insufficiency
Toxic	Lead
	Iron
	Vitamin A and D
	Medications—ipecac, digoxin, theophylline, etc.
Cardiac	Congestive heart failure
	Vascular ring
Others	Pediatric falsification disorder (Munchausen syndrome by proxy)
	Child neglect or abuse
	Self-induced vomiting (rumination syndrome)
	Cyclic vomiting syndrome
	Autonomic dysfunction

Adapted from Vandenplas et al. [1]

symptoms of GERD (Table 10.2). The clinician needs to be aware that not all regurgitation and vomiting in infants and young children is related with GERD. Bilious vomiting, gastrointestinal bleeding, consistently forceful vomiting, weight loss or failure to thrive, diarrhea, constipation, fever, lethargy, hepatosplenomegaly, abdominal tenderness, or distension should raise the possibility of an alternate diagnosis. Bulging fontanelle, macro- and/or microcephaly, and seizures raise the possibility of genetic and/or metabolic syndromes.

Belching or eructation occurs during a TLESR and is an important method of venting air from the stomach. Hiccups

are involuntary reflex contractions of the diaphragm followed by laryngeal closure. In some cases, hiccups cause or are related to GER.

Atypical symptoms such as epigastric pain, nausea, flatulence, hiccups, chronic cough, asthma, and hoarseness account for 30–60% of presentations of GERD [1, 2] (Table 10.1). Possible associations exist between GERD and asthma, pneumonia, bronchiectasis, brief resolved unexplained events (BRUEs), laryngotracheitis, and sinusitis, but causality or temporal association was not established, except for dental erosions and Sandifer syndrome [2, 5].

GER and Uncomplicated Regurgitation

Regurgitation is the most common presentation of infantile GER, with occasional projectile vomiting.

Over 50% of healthy infants have regurgitation that is physiologic, often decreasing from the age of 6 months onward and resolving without intervention in 95% of individuals by 12–18 months of age [2, 8] (Fig. 10.3). Daily regurgitation occurs more frequently in infants during the first 6 months of life than in older infants and children. Frequent regurgitation, defined as ≥4 times per day, occurs in about 25% of infants during the first months of life. About 20–25% of parents seek medical advice because of frequent infantile regurgitation, which corresponds to at least four episodes of regurgitation a day [2, 8]. Rome IV criteria consider >2 episodes of regurgitation per day during more than 3 weeks as a functional gastrointestinal disorder [11]. Functional gastrointestinal disorders are most of the time discussed separately although up to 75% of infants present with a combination of functional disorders, and not with just one [48].

A prospective follow-up study reported disappearance of regurgitation in all subjects before 12 months, although an increased prevalence of feeding refusal, duration of meals, parental feeding-related distress, and impaired quality of life was observed up to the age of 3 years, even after disappearance of symptoms [49]. Infants with spilling 90 days or more during the first 2 years of life, classified as frequent spilling, are more likely to have GER symptoms at 9 years of age [50]. Excessive regurgitation is one of the symptoms of GERD, but the terms excessive regurgitation and GERD should not be used as synonyms.

Although most studies report a comparable incidence of regurgitation in unselected populations of formula versus breastfed infants, Hegar et al reported a higher incidence in formula-fed infants [8]. This observation fits with the knowledge that GER and symptoms of GERD may be indistinguishable from those of food allergy [2, 5]. The incidence of cow milk protein allergy is 5–10 times higher in formula fed than in breastfed infants [51].

Although the "happy spitter" certainly exists and is not rare, many infants show some symptoms of distress and discomfort when regurgitating. Irritability and distress may accompany regurgitation and vomiting; however, in the absence of other warning symptoms, it is not an indication for extensive testing [1, 2]. Parental carrying-capacity or anxiousness ("parental coping") will determine if a physician is contacted by anxious parents or not. Infant regurgitation is a benign condition with a good prognosis, needing no other intervention than parental education and anticipatory guidance, and intervention on feeding frequency, volume, and composition will contribute to parental reassurance [1, 2]. Regurgitation is almost never a reason to stop breastfeeding. In formula-fed infants, thickened formula will alleviate regurgitation and promote parental reassurance. Overfeeding

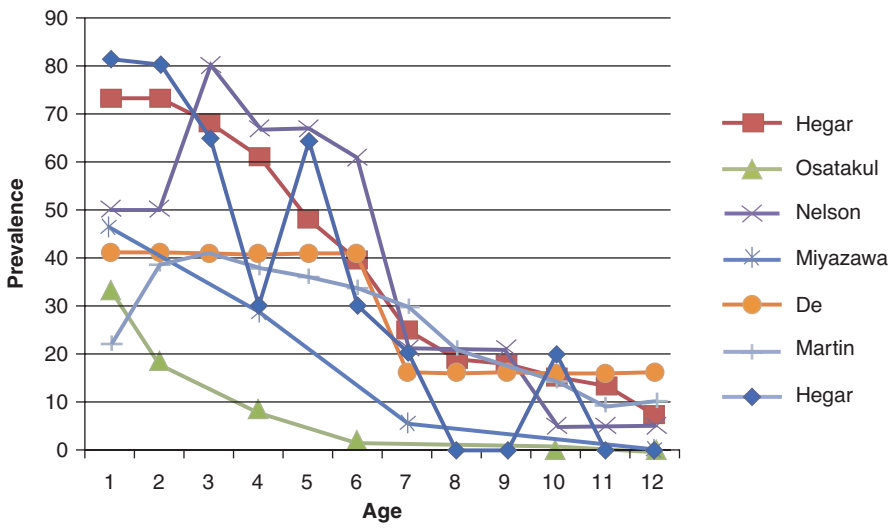


Fig. 10.3 Natural evolution of physiologic regurgitation. (Adapted from Ref. [10])

exacerbates recurrent regurgitation. Thickened or anti-regurgitation formula decreases overt regurgitation [1, 2].

GER(D) and Recurrent Regurgitation and Poor Weight Gain

If failure to thrive is documented, it is obvious that the infant is not a happy spitter. Poor weight gain is a crucial warning sign that necessitates clinical management. These infants need a full diagnostic workup starting with a dietary history to evaluate caloric intake. Hospitalization of these infants may be needed. Although regurgitation usually causes little more than a nuisance, very important regurgitation may induce caloric insufficiency and malnutrition. These infants are sometimes diagnosed as suffering nonorganic failure to thrive, a disorder that sometimes is attributed to social/sensory deprivation, socioeconomic, or maternal-child relation problems. GERD is only one of the many etiologies of "feeding problems" in infancy. Poor weight gain, feeding refusal, back-arching, irritability, and sleep disturbances have been reported to be related as well as unrelated to GERD [2, 52].

GER(D) and Cow's Milk Protein Allergy

Guidelines on the symptoms and management of cow's milk protein allergy (CMPA) unanimously report that persistent regurgitation and vomiting can possibly be manifestations of CMPA (Table 10.4: overlapping symptoms between GER(D) and CMPA) [2, 53]. The positive response to cow's milk protein elimination from the diet and relapse of symptoms is the cornerstone of the diagnosis of CMPA. An association between GERD and cow milk hypersensitivity was observed in both infants and children with severe GERD [54]. Simultaneous cow milk challenge and pH monitoring had limited value as a method to identify this subgroup [54]. Impedance has shown that the incidence of nonacid post-prandial reflux in decreased after a feeding with an amino acid-based formula compared to standard infant formula in these infants [34]. However, since amino acids or extensive hydrolysates have much more rapid gastric emptying than standard infant formula with intact cow milk proteins [55, 56], it is not possible to know if the decrease in GER is due to the enhanced gastric emptying or to an autoimmune mechanism.

Table 10.4 Signs and symptoms of GER(D) and CMPA

GER(D)	GER(D)+/- CMPA	CMPA
Aspiration	Apnea/ALTE/SIDS	Anaphylaxis
Back-arching	Colic	Angioedema
Bradycardia	Constipation	Bloody stools
Dysphagia	Failure to thrive	Diarrhea
Hematemesis	Feeding refusal	Eczema/dermatitis
Hiccups	Irritability	Itching
Hoarseness	Parenteral anxiety	Lip swelling
Laryngitis/stridor	Regurgitation	Nasal congestion
Melena	Sleep disturbances	Rhinitis
Nausea/belching	Vomiting	Urticaria
Respiratory infections	Wheezing	
Rumination		
Sandifer's syndrome		

Adapted from: Salvatore and Vandenplas [272]

GERD and Esophagitis

Reflux esophagitis is reported to occur in 2–5% of the population. Esophagitis is defined as visible breaks of the esophageal mucosa [1, 2]. Histology is recommended to rule out Barrett's esophagus when suspected during upper GI endoscopy or to exclude other causes of symptoms such as eosinophilic esophagitis. Children with GER symptoms present esophagitis in 15–62%, Barrett's esophagus in 0.1–3%, and refractory GERD requiring surgery in 6–13% [1, 2, 45, 57]. In comparison, esophagitis is observed in only 20–40% of adults with heartburn, and Barrett's esophagus in 1.8–4% [58–63].

Hiatal hernia is more frequent in children with erosive esophagitis than without (7.7% versus 2.5%) [64]. Erosive esophagitis in 0- to 17-year-old children with GERD symptoms was reported to be 12.4% increasing with age [64]. The median age of the group with erosive esophagitis was 12.7 ± 4.9 years versus 10.0 ± 5.1 years in those without [64]. The incidence of erosive esophagitis was only 5.5% in those younger than 1 year [64]. The differences in incidence of esophagitis are determined by patient recruitment, differences of definition of esophagitis, and the availability of self-treatment. With an incidence of nearly 25%, acid-blocking medication is likely to be overprescribed to extremely low birth weight infants at the moment of discharge [65].

Esophagitis, identified by histology, occurs in 61–83% of infants with reflux symptoms severe enough to perform endoscopy. Although esophagitis may present with pain, it can also be asymptomatic. The group with asymptomatic esophagitis is in some ways the most problematic. Even severe esophagitis may remain asymptomatic as demonstrated by children who present with peptic strictures without having experienced any discomfort attributable to esophagitis. Patients with Barrett's esophagus can be almost symptom free. The more severe the lesions, the more nerve endings are damaged or destroyed. Typical substernal burning pain (heartburn, pyrosis) occurs in many children suffering from esophagitis. Odynophagia, which is pain on swallowing, usually represents esophageal inflammation. In nonverbal infants, behaviors suggesting esophagitis include crying, distress, irritability, sleep disturbance, and "colic." Infants frequently also appear very hungry for the bottle until

their first swallows and then become irritable and refuse to drink. Dysphagia, typical for eosinophilic esophagitis, has also been linked to esophagitis.

GER(D) and Eosinophilic Esophagitis

The impressive rise in prevalence of eosinophilic esophagitis (EoE) is still poorly understood [65, 66]. Especially in young infants, distinguishing EoE from reflux esophagitis may be difficult. In reflux esophagitis, the eosinophilic infiltrate is in theory limited to less than 5/per high power field (HPF) compared to primary EoE with >15 eosinophils per HPF. More recently, failure of PPI treatment as a condition to diagnose EoE brought reflux esophagitis back in the picture of EoE [67]. EoE necessitates proper treatment (hypoallergenic feeding, corticoids, montelukast, etc.). Patients with allergic esophagitis are often younger and have atopic features such as allergic symptoms or positive allergic tests, but have often no specific symptoms. Atopic features are reported in more than 90% and peripheral eosinophilia in up to 50% of patients, but of course depending on selection of patients. At endoscopy, a pale, granular, furrowed, and occasional ringed esophageal mucosa may appear [2]. While symptoms in older children are more oriented to dysphagia for solids, symptoms in infants are more reflux-like [68, 69]. Repeated endoscopy with esophageal histology in combination with response to treatment may in some cases be the only way out to separate reflux esophagitis from EoE in young children. The cornerstone of treatment is an elimination diet (targeted or empiric elimination diet, amino acid-based formula) and/or swallowed, topical corticosteroids [67]. Systemic corticosteroids are reserved for severe symptoms requiring rapid relief or where other treatments have failed [66]. Significant differences in general practice between pediatric and adult gastroenterologist were demonstrated with notable divergence from consensus guidelines [70]. Although elimination diets remain an appropriate option, the vast majority of adults suffering from EoE will be started on topical corticosteroids for long-term management, mainly as a result of the poor long-term compliance to elimination diets [71]. International practice variations are also apparent. An in-depth discussion on EoE is beyond the scope of this chapter.

GER(D) and Heartburn, Infant Crying, and Distressed Behavior

While the verbal child can communicate pain, descriptions of the intensity, location, and severity may be unreliable until the age of at least 8–12 years [5]. In adults, adolescents, and older children, heartburn and regurgitation are the characteristic manifestations of the reflux syndrome [6]. GERD in adolescents is more adult-like. Heartburn is a symptom of GERD with or without esophagitis. Heartburn is a predominant GER symptom in adults, occurring weekly in 15–20% and daily in 5–10% of subjects [72, 73]. Diagnosis and management of GERD in older children (>12 years) and adolescents follows the recommendations for adults [2]. According to parents, heartburn is present in 1.8% of 3- to 9-year-old healthy children and 3.5% of 10- to 17-year-old adolescents; regurgitation is said to occur in 2.3% and 1.4%, respectively, and 0.5% and 1.9% need antiacid medication [50]. According to self-reports, adolescents complain about heartburn in 5.2% and regurgitation up to the pharynx in 8.2%, while antiacids are taken by 2.3% and histamine receptor antagonists (H_2RA) by 1.3%, suggesting that symptoms of GER are not rare during adolescence and are underreported by parents or overestimated by adolescents [50]. In infants, the issue is more complicated. Since per definition "heartburn" suggests that the individual with heartburn feels a burning retrosternal pain, parents and healthcare providers almost automatically hypothesize that a "crying baby" or a "distressed baby" is likely to suffer from heartburn or "occult GER." As a consequence, acid-reducing medication is increasingly prescribed in infants [74, 75]. Several randomized controlled trials were performed for this indication, and for once all results come to the same conclusion: proton-pump inhibitors are useless to decrease crying and distressed behavior in newborns and infants [75]. Heine and coworkers identified no relation between crying duration and the result of pH monitoring [10]. In other words: many infants that regurgitate are distressed and cry, but only very few infants presenting with distressed behavior or crying without regurgitation suffer GER(D). Occult GER in infants is almost nonexisting.

The same amount of distress and crying may be evaluated by some parents as easily acceptable while it will be unbearable for other parents. In fact, the coping capacity of the parents decides if medical help is looked for. Many factors, such as tobacco smoke, may cause infant irritability. CMPA is another well-identified cause of infant irritability. There is substantial individual variability, and some healthy infants may cry up to 6 h a day [1, 2].

The concept that infant irritability and sleep disturbances are manifestations of GER is largely derived from adult data [1, 2]. In adults, sleep disturbance attributable to GERD symptoms has been demonstrated, with a beneficial effect of PPI [76, 77]. The developing nervous system of infants exposed to acid seems susceptible to pain hypersensitivity despite the absence of tissue damage. In adults, NERD is an accepted entity as it is the most frequent presentation of GERD. In adults, impaired quality of life, notably regarding pain, mental health, and social functioning, has been demonstrated in patients with GERD, regardless of the presence of esophagitis [78]. In

an unselected population, 28% of the adults report heartburn, almost half of them weekly, with a significant impact on the quality of life in 76%, especially if the symptoms are frequent and long lasting. Despite that, only half of the heartburn complainers seek medical help, although 60% takes medication [79]. Thus, some adults "learn to live with their symptoms" and acquire tolerance to long-lasting symptoms. In infancy and young children, verbal expression of symptoms is often vague or impossible, and persistent crying, irritability, back-arching, feeding, and sleeping difficulties have been proposed as possible equivalents of adult heartburn. Infants with GERD learn to associate eating with discomfort and thus subsequently tend to avoid eating and develop an aversive behavior around feeds, although behavioral feeding difficulties are also common in control toddlers [80]. Esophageal pain and behaviors perceived by the caregiver to represent pain potentially affect the response of the infant to visceral stimuli and the ability to cope with these sensations, both painful and nonpainful. A placebo-controlled randomized trial with proton-pump inhibitors in distressed infants showed an equal decrease in distressed behavior in the treatment and the placebo group [81]. Up to date, there is no evidence that acid-suppressive therapy is effective in infants who present solely with inconsolable crying or distressed behavior. Moreover, inappropriate administration of acid-blocking medication in infants should be avoided as this medication often causes adverse effects. In infants and toddlers, there is no symptom or group of symptoms that can reliably diagnose GERD or predict treatment response. Infants presenting with isolated distressed behavior or crying should get appropriate care, but acid GERD not resulting in regurgitation or vomiting but causing excessive crying and distress is exceptional.

GER(D) and Dysphagia, Odynophagia, and Food Refusal

Dysphagia is the difficulty of swallowing; odynophagia is pain caused by swallowing. Although GERD is frequently mentioned as a cause of dysphagia or odynophagia, there are no pediatric data showing this relation. Dysphagia is a prominent symptom in patients with EoE. Feeding difficulty and/or refusal are often used to describe uncoordinated sucking and swallowing, gagging, vomiting, and irritability during feeding. Thickeners are effective in improving swallowing mechanics [82]. A relation between GER, GERD, and feeding refusal has not been established. In case of acute feeding difficulties, a trapped foreign body should be among the list of possible differential diagnoses. In case of chronic feeding difficulties, achalasia should be considered.

GER(D) and Extra-Esophageal Manifestations

Although there is sufficient evidence to support an association between extra-esophageal symptoms and GERD, there is no evidence for a causal relationship. Laryngopharyngeal symptoms of GERD such as globus sensation, hoarseness, and chronic cough are becoming increasingly recognized. In the pediatric literature, little attention has been given to globus pharyngeus sensation, probably related to the fact that young children will experience difficulties to express this sensation. There is no evidence that medical treatment reduces extra-esophageal manifestations. Pulmonary microaspiration as demonstrated by pepsin detection in a bronchoalveolar lavage fluid is common in children with chronic lung diseases, suggesting that GER may contribute significantly to the disease pathogenesis [83]. The bronchoalveolar lavage pepsin concentration correlates positively with the number of proximal reflux events [83]. Protein oxidation in the bronchoalveolar lavage is higher in children with extensive proximal acidic reflux, suggesting that pulmonary microaspirations contribute to lung damage [83]. Children >1 year with GERD-related respiratory symptoms showed a significantly higher number of weakly alkaline refluxes than children with GERD-related GI symptoms [84]. This supports the hypothesis that respiratory symptoms are less related to acidity than GI symptoms [84].

GER(D) and Reactive Airway Disease

An etiologic role for GER in reactive airway disease has not been demonstrated, but the association between GERD and asthma is bidirectional: the asthma group has a 1.36 higher risk for GERD, and the GERD group has a 1.48 higher risk for asthma [85]. Prior severe asthma exacerbations (incidence rate ratio 3:45) and younger age increased the severe asthma exacerbation risk in all countries, whereas obesity, atopy, and GERD were a risk factor in some but not all countries. Rehospitalization rates were up to 79% within 1 year [86].

Different pathophysiologic mechanisms are proposed: direct aspiration, vagal mediated bronchial and laryngeal spasm, neural mediated inflammation. Esophageal acidification in infants with wheezing can produce airway hyperresponsiveness and airflow obstruction [87]. Few studies tempted to evaluate the opposite: the impact of asthma on the severity of GERD. Chronic hyperinflation as occurs in asthma favors many GER mechanisms. An association between asthma and reflux measured by pH or impedance probe has been reported in many studies [2]. Wheezing appears more related to GERD if it is nocturnal. A recent study reports a high prevalence of GER in children and adolescents with persistent asthma, equally distributed in the supine nocturnal and upright positions [88]. But there was no

correlation between the result of the pH metry and pulmonary function tests [88].

Very few prospective, randomized, and blinded treatment studies have been performed in children. In a series of 46 children with persistent moderate asthma despite bronchodilators, inhaled corticosteroids, and leukotriene antagonists, 59% (27/46) had an abnormal pH metry [89]. Reflux treatment did result in a significant reduction in asthma medication. Patients with a normal pH metry were randomized to placebo or reflux treatment: 25% (2 of only 8 children) of the treated patients could reduce their asthma medication, while this was not possible in any patient on placebo [89]. Another study found omeprazole ineffective in improving asthma symptoms and parameters in children with asthma [90]. Overall, although there seems to be an association between GER and asthma, the causal role of GER has not been demonstrated. There is no association between asthma control status and laryngopharyngeal reflux and GER [91]. Current evidence does not support the routine use of anti-GERD medication in the treatment of poorly controlled asthma of childhood [92]. Selection of patients is once more of importance. Children with asthma and heartburn should be treated with acid-reducing medication because of the heartburn, not because of the asthma. Many studies in children with extra-esophageal symptoms included children that also presented with typical reflux symptoms.

GER(D) and Recurrent Pneumonia

The reported mechanisms are similar to those for reactive airway disease. Direct aspiration during swallowing may be more relevant in this group. No test can determine whether reflux is causing recurrent pneumonia. Upper esophageal and pharyngeal pH and impedance recordings provided contradictory information. A new technique to record pharyngeal reflux had been developed (Restech→) [93]. However, results could not confirm the utility of this technique [94]. As a consequence, pharyngeal pH recording is no longer used.

Lipid-laden macrophages have been used as an indicator of aspiration, but their sensitivity and specificity for GER is poor. One study evaluating nuclear scintigraphy with late imaging reported that 50% of patients with a variety of respiratory symptoms had pulmonary aspiration after 24 h [95]. However, later studies failed to reproduce these findings [96]. Aspiration also occurs in healthy subjects, especially during sleep [1]. Weakly acid reflux events can be associated with a significant airway inflammation and injury that, because of the biochemical mechanisms involved, are likely not completely preventable and/or counteracted by antiacid treatments [97].

The role of reflux in patients with bronchopulmonary dysplasia and other chronic respiratory disorders is not clear. Today, the clinician has frequently no other option than to make management decisions based on inconclusive diagnostic studies with no certainty regarding outcome [1, 2]. As in reactive airway disease, it is very likely that nonacid reflux can cause airway manifestations.

GER(D) and Cystic Fibrosis

The incidence of GER in children with cystic fibrosis is very high. Excessive acid reflux exists in the majority of cystic fibrosis patients, even before respiratory symptoms develop [2, 98, 99]. CF patients also suffer from duodenogastroesophageal reflux of bile acids [100, 101]. In the majority of patients, typical GER symptoms are absent [99]. Therefore, diagnostic procedures should be considered, regardless of lacking symptoms. Patients with cystic fibrosis have a relatively high number of proximal reflux episodes [99]. Such episodes also indicate an increased risk for aspiration. It is possible that as well the acid and bile reflux are aggravating the respiratory symptoms, and that the respiratory symptoms aggravate the reflux. Aggressive medical and surgical reflux treatment in this patient group seems reasonable. In children with cystic fibrosis, a better weight gain was reported during PPI treatment; whether this is due to a reduction of acid reflux or better buffering of acid gastric content in the intestine is not clear. Almost half of the children with cystic fibrosis and symptoms suggestive for GERD have increased acid GOR and almost a quarter has delayed gastric emptying [100]. However, there is no relation between GOR and gastric emptying [100]. Lung transplantation exacerbates gastroesophageal reflux disease [102]. Reflux burden and fundoplication status do not impact lung transplant outcomes, but gastric dysmotility may be linked to allograft dysfunction in children [103]. An association between GERD and allograft injury was reported, encouraging a strategy of early diagnosis and aggressive reflux management in lung transplant recipients to improve transplant outcomes [83, 104].

GER(D) and Cough and ENT Manifestations

Both acid and weakly acid GER may precede cough in children with unexplained cough, but cough does not induce GER [105]. Objective cough recording improves symptom association analysis. Treatment for GERD should not be used when there are no clinical features of GERD, and pediatric GERD guidelines should be used to guide treatment and investigations [106]. Several studies revealed the presence of pepsin in the middle ear fluid, but with a huge variation in incidence varying from 14% to 73% [2, 107]. Pepsin in saliva appears to be associated with laryngomalacia, suggesting a role for salivary pepsin as a noninvasive marker of laryngopharyngeal reflux in patients with laryngomalacia [108]. Recent data showed that salivary pepsin to detect GERD is not ready for clinical application [109]. Also, bile acids have

been detected in middle ear liquid, even in higher concentrations than in serum [110]. The presence of pepsin and bile in middle ear fluid might as well be the consequence of reflux and vomiting at the moment of the acute middle ear infection than an argument to hypothesize that chronic GER may be at the origin of the chronic middle ear problem. Several epidemiologic studies suggest a low incidence of reflux symptoms in patients with recurrent middle ear infections.

Data suggesting a causal relation between reflux and upper airway disease in children are limited. Data from several placebo-controlled studies and meta-analyses uniformly have shown no effect of antireflux therapy on upper airway symptoms or signs [2]. Well-designed, prospective, placebo-controlled, blinded studies are needed. Another bias might be selection of patients: these studies are frequently set up in tertiary care centers in highly selected patient populations. The question is how representative these patients are for the bulk of children with upper respiratory and/or ENT manifestations.

GER(D) and Dental Erosions

Young children and children with neurologic impairment appear to be at greatest risk to have dental erosions caused by GER. Juice drinking, bulimia, and racial and genetic factors that affect dental enamel and saliva might be confounding variables that have been insufficiently considered [1, 2]. A positive correlation between mainly acid GERD and dental erosion has been as well confirmed as refuted, although the evidence for a relation between both seems to win the debate [110–113]. There are no long-term (intervention) follow-up studies in high-risk populations.

GER(D) and Sandifer Syndrome

Sandifer syndrome is an uncommon but specific manifestation of GERD. The presenting symptoms of Sandifer's may include any combination of abnormal movements and/or positioning of head, neck, trunk, and upper limbs, seizure-like episodes, ocular symptoms, irritability, developmental and growth delay, and iron-deficiency anemia [114]. Successful treatment of the underlying GERD led to a complete or near-complete resolution of the neurological symptoms in all of the reviewed cases [114].

GER(D) in Neurologically Impaired Children

Neurologically impaired children have more frequent, more severe, and more difficult to treat GERD than neurologically normal children. An ESPGHAN Working Group published a consensus statement on the diagnosis and management of GERD in neurologically impaired children [115]. Neurologically impaired children accumulate many risk factors for severe GERD: spasticity or hypotonicity, supine position, constipation, etc. Diagnosis of reflux disease in these children is often difficult because of their underlying conditions. Empiric treatment will often be initiated. Whether this group of patients has more severe reflux disease, or has less effective defense mechanisms, or presents with more severe symptoms because of the inability to express and/or recognize symptoms remains open for debate. Response to treatment, both medical and surgical, was reported to be poor in the neurologically impaired child compared to the neurologically normal child. However, surgical experience for multiple centers reports different experience with no differences in outcome related to neurological impairment [116–118]. Gastrostomy feeding may reduce aspiration but could exacerbate gastroesophageal reflux disease [115]. The impact of antireflux procedures in addition to gastrostomy is relatively unknown [115].

GER(D) and Apnea, Brief Resolved Unexplained Events, and Sudden Infant Death Syndrome

In adults, a relation between obstructive sleep apnea and GERD has been demonstrated [119, 120]. It is commonly assumed that nightly GER events causes sleep disturbance by arousal. However, a study combining polysomonography and pH/impedance monitoring challenged this hypothesis [121]. Literature can best be summarized as follows: series fail most of the time to show a temporal association between GER and pathologic apnea, apparent life-threatening events, and bradycardia [1, 122]. The term "brief resolved unexplained event" (BRUE) was created to replace "apparent life-threatening event," narrowing the definition and providing evidence-based guidelines for management. There are well-selected cases or small series that demonstrate that pathologic apnea can occur as a consequence of GER. A relation between GER and short, physiologic apnea has been shown [123]. GER is a frequent cause of interrupting sleep in infants, and nonacid GER is equally important as acid GER for causing arousal and awakening in infants [124, 125]. GER events preceded cardiorespiratory events in 83% of these associations [82, 99]. These GER events had a higher proximal extent [124]. Discomfort is significantly associated with reflux events and does not differ between weakly acidic and acid refluxes [126]. In general, GER is not related to pathologic apnea, significant bradycardia, and BRUE, but exceptions do exist [122]. Gastroesophageal reflux can cause sudden death in a vulnerable infant during a critical period of development through failure of "autoresuscitation" mechanisms [127].

GER(D) and Other Risk Groups

There are no data in literature that preterm babies have more (severe) reflux than term born babies, although many preterms are treated for reflux.

Symptomatic GER is extremely frequent in patients operated because of esophageal atresia and/or tracheoesophageal fistula because of serious structural and functional deficiencies [128]. The reflux in these children might be refractory to medical treatment. The high rates of wrap failure necessitate close follow-up [128].

Children with congenital abnormalities or after major thoracic or abdominal surgery are at risk for developing severe GERD.

GERD and Complications

Children with neurological impairment, chronic lung disease (especially cystic fibrosis), esophageal atresia, and chemotherapy have the most severe pathologic reflux and are at high risk for the development of complications of GERD [1, 2].

Barrett's esophagus, esophageal strictures, and adenocarcinoma are complications of chronic severe GERD. Barrett's esophagus is a premalignant condition in which metaplastic specialized columnar epithelium with goblet cells replaces the squamous epithelium of the esophagus. Although Barrett's esophagus is considered a premalignant condition, the incidence of carcinoma in pediatric population remains low [129]. Differences in esophageal mucosal resistance and genetic factors may partially explain the diversity of lesions and symptoms. Patients with esophageal atresia are at high risk of persisting GERD and Barrett's esophagus [130]. The development of Barrett's esophagus is related to GERD history. As Barrett's esophagus represents a premalignant condition, long-term systematic follow-up of the esophageal mucosa including multistaged biopsies is warranted, even in asymptomatic patients [130].

More than 60 years ago, in the absence of acid-blocking medication, esophageal strictures were reported in about 5% of children with reflux symptoms [131]. Currently, esophageal stenosis and ulceration in children have become rare. In a series including 402 children with GERD without neurological or congenital anomalies, no case of Barrett's esophagus was detected [57]. In another series including 103 children with long-lasting GERD, and not previously treated with a H_2 receptor antagonist (H_2RA) or a proton-pump inhibitor (PPI), Barrett's esophagus was detected in 13%. An esophageal stricture was present in 5 of the 13 patients with Barrett [132]. Reflux symptoms during childhood were not different in adults without or with Barrett [133]. Barrett has a male predominance, and increases with age. Patients with short segments of columnar-lined esophagus and intestinal metaplasia have similar esophageal acid exposure but significantly higher frequency of abnormal bilirubin exposure and longer median duration of reflux symptoms than patients without intestinal metaplasia [134]. There is a genetic predisposition in families in patients with Barrett's esophagus and esophageal carcinoma [1].

Peptic ulcer, esophageal, and gastric neoplastic changes in children are seldom observed. In adults, over the last 30 years, a decreased prevalence of gastric cancer and peptic ulcer with an opposite increase of esophageal adenocarcinoma and GERD has been noted. This has been attributed to independent factors among which changes in dietary habits such as a higher fat intake, an increased incidence of obesity, and a decreased incidence of *H. pylori* infection. The incidence of noninvasive in situ cancer has actually declined after 2003 [135]. Frequency, severity, and duration of reflux symptoms are related to the risk to develop esophageal cancer. Among adults with long-standing and severe reflux, the odds ratios are 43.5 for esophageal adenocarcinoma and 4.4 for adenocarcinoma at the cardia [136]. It is unknown whether mild esophagitis or GER symptoms persisting from childhood is related to an increased risk for severe complications in adults.

Diagnosis

It is beyond the scope of the subject to provide a detailed discussion on all diagnostic procedures for GERD. Detailed information regarding indications and pitfalls of radiologic contrast studies, nuclear reflux scintigraphy, ultrasound, endoscopy, manometry, gastric emptying tests, and electrogastrography can be found in review papers and guidelines [1, 2]. The applicability of the recent Lyon consensus for diagnosing GERD in adults needs to be discussed for its applicability in pediatrics [137]. According to the Lyon consensus, conclusive evidence for reflux on esophageal testing include advanced grade erosive esophagitis (LA grades C and D), long-segment Barrett's mucosa or peptic strictures on endoscopy, or distal esophageal acid exposure time (previously called "reflux index") >6% on ambulatory pH or pH-impedance monitoring "off" PPI [137]. A normal endoscopy does not exclude GERD, but provides supportive evidence refuting GERD in conjunction with distal acid exposure time <4% and <40 reflux episodes on pH-impedance monitoring off proton-pump inhibitors [137]. As a consequence, an acid exposure time between 4% and 6% is considered "gray zone". Reflux-symptom association on ambulatory reflux

monitoring provides supportive evidence for reflux-triggered symptoms, and may predict a better treatment outcome when present. When endoscopy and pH or pH-impedance monitoring are inconclusive, adjunctive evidence from biopsy findings (histopathology scores, dilated intercellular spaces), motor evaluation (hypotensive lower esophageal sphincter, hiatus hernia, and esophageal body hypomotility on high-resolution manometry), and novel impedance metrics (baseline impedance, post-reflux swallow-induced peristaltic wave index) can add confidence for a GERD diagnosis; however, diagnosis cannot be based on these findings alone [137]. An assessment of anatomy, motor function, reflux burden, and symptomatic phenotype will therefore help direct management. Future GERD management strategies should focus on defining individual patient phenotypes based on the level of refluxate exposure, mechanism of reflux, efficacy of clearance, underlying anatomy of the esophagogastric junction, and psychometrics defining symptomatic presentations. The Lyon Consensus goes beyond the previous classifications and defines endoscopic and functional parameters able to establish the presence of GERD [138]. The "Lyon Consensus" panel of experts confirmed that positive indices of reflux-symptom association, without other altered parameters, represent reflux hypersensitivity. GERD requires a customized management; it is crucial to assess frequency and severity of symptoms and their response to an optimal course of therapy as well as to explore the endoscopic alterations and consider other diagnoses responsible for persistent symptoms [139]. The Lyon consensus will contribute to diagnose GERD objectively, but seems not very useful for primary health care.

In adults, the diagnosis of GERD mainly relies on clinical history. Heartburn and acid regurgitation are considered the cardinal symptoms of GERD [137]. However, history in young children is considered poorly reliable up to the age of minimally 8 or even 12 years old. Therefore, questionnaires have been developed trying to improve history reliability. Orenstein developed the "infant GER-questionnaire" (I-GER) [140]. The questionnaire results in an objective, validated, and repeatable quantification of symptoms suggesting GERD. The I-GER was revised (the "I-GERQ-R") in 185 patients and 93 controls, resulting in an internal consistency reliability from 0.86 to 0.87, and a test-retest reliability of 0.85 [141]. However, according to data from Aggarwal and coworkers, the I-GER-Q has a sensitivity of only 43% and a specificity of 79% [96]. Moreover, pH metry results were not different according to a "positive" or "negative" score of the I-GER-Q [142]. Our group showed that not one question was found to be significantly predictive for the presence of esophagitis. In our hands, the Orenstein I-GERQ cut-off score failed to identify 26% of infants with GERD according to pH metry results or presence of esophagitis, but was positive in not less than 81% of infants with a normal histology of esophageal biopsies and normal pH metry [143]. Deal et al. developed two different questionnaires, one for infants and one for older children, and showed that the score was higher in symptomatic than in asymptomatic children [144]. Overall, it can be concluded that the correlation between the results of history obtained by questionnaires and of reflux investigations is poor.

Barium contrast radiography, nuclear scintiscanning, and ultrasound are techniques evaluating postprandial reflux and provide limited information on gastric emptying and pathologic GER. Normal ranges are not established for any of these procedures. Barium studies are not recommended as first-line investigation to diagnose GERD, but are of importance to diagnose anatomic abnormalities such as malrotation, duodenal web, and stenosis and may suggest functional abnormalities such as achalasia.

Nuclear scintigraphy may show pulmonary aspiration [95]. However, these findings could not be confirmed [96]. Aspiration of saliva and gastric contents occurs during sleep in healthy adults. Scintigraphy can estimate gastric emptying. But the 13C-octanoic acid (for solids) and 13C-acetate (for liquids) breath tests are more appropriate to measure gastric emptying in children [145, 146]. A putative role of delayed gastric emptying in GER(D) was raised by studies in the pre-PPI era, but its importance in the pathophysiology of GERD has been questioned ever since [147].

In the hands of an experienced investigator, ultrasound provides morphological and functional data with high sensitivity and positive predictive value for the diagnosis of GER [148]. Sonographic assessment of findings such as abdominal esophageal length, esophageal diameter, esophageal wall thickness, and gastroesophageal angle provide important diagnostic indicators of reflux and related to the degree of GER [148]. However, there is a need for standardization of the procedure and for defining diagnostic criteria [148]. The results of ultrasound are investigator dependent, and a relation between reflux seen on ultrasound and symptoms has not been established [1, 2]. Overall, ultrasound may detect GER but is not sensitive or specific to diagnose GERD. There is no indication for electrogastrography in the diagnostic workup of a patient suspected of GERD.

Endoscopy allows direct visual examination of the esophageal mucosa. Modern endoscopes are so miniaturized that upper GI endoscopy of preterm infants of less than 1,000 g has become technically easy. Operator experience is an important component to guarantee interobserver reliability [1, 2]. Macroscopic lesions associated with GERD include esophagitis, erosions, exudate, ulcers, strictures, hiatal hernia, etc. Redness of the distal esophagus in young infants is a normal observation because of the increased number of small blood vessels at the cardiac region. Endoscopy may also show a "sliding hernia," the stomach that is protruding in the esophagus especially during burping. Recent consen-

sus guidelines define reflux esophagitis as the presence of endoscopically visible breaks in the esophageal mucosa at or immediately above the GE junction [1, 2]. These mucosal breaks are classified according to the Los Angeles classification [149]. Endoscopy-negative reflux disease is common. There is a poor correlation between the severity of symptoms and presence and absence of esophagitis. Especially in infants, nonacid reflux was shown to cause at least as much distress as acid reflux. There is insufficient evidence to support the use of histology to diagnose or exclude GERD. However, biopsies of duodenal, gastric, and esophageal mucosa are mandatory to exclude other diseases [1]. More detailed information on pros and cons of histology can be found in the recent consensus papers [1, 2].

Esophageal manometry does not demonstrate reflux, but is of interest to analyze pathophysiologic mechanisms causing reflux, mainly by visualizing and measuring TLESRs, and is indicated in the diagnosis of specific conditions such as achalasia. With the avenue of solid-state catheters and high-resolution manometry, the pressure topography of the full length of the esophagus can be achieved [150]. In adults, high-resolution esophageal manometry should always be considered before antireflux surgery [151]. Ambulatory 24-h esophageal manometry, in combination with pH metry and/or impedance recording, is nowadays technically feasible. This technique is mainly used in (clinical) research and allows the objective demonstration of reflux-symptom association (e.g., in patients presenting with chronic cough).

Intraluminal esophageal acid perfusion provoking chest pain (Bernstein test) or using other end-points has found expanded use in practice and research in the USA, but has never been popular in Europe.

Ambulatory 24-h esophageal pH monitoring measures the incidence and duration of acid reflux, while impedance measures all types of reflux episodes, independent of pH. The technique offers the possibility to measure intragastric and esophageal pH simultaneously in older children. Both hardware (electrodes, devices) and software influence the results [1]. Esophageal pH metry is the best method to measure acid in the esophagus, but not all reflux causing symptoms is acid and not all acid reflux is causing symptoms. The cut-off of pH 4.0 was historically determined as the best cut-off in adults with heartburn. This cut-off was taken over for children, without ever testing if this was the best cut-off. Moreover, it is very unlikely that a pH of 4.1 or 3.9 has a different clinical meaning. Therefore, it is a shortcoming that modern analysis programs do no longer calculate the oscillatory index, which calculates the % oftie that the pH oscillates between 3.75 and 4.25 [152]. The oscillatory index provides information on the risk of erroneous interpretation of the pH metry [152]. The surface area below pH 4.0 was shown to correlate well with histologic esophagitis, while the reflux index, the % of time with pH <4.0 does not [153]. Esophageal pH metry is useful in evaluating the effect of a therapeutic intervention on reducing esophageal acid exposure. Medical treatment is focusing on the reduction of gastric acid secretion. Normal ranges have been established for pH metry. However, normal ranges depend also on the hard- and software used. The major indication of long-term recording of pH and/or impedance is the demonstration of an association between reflux and symptoms.

Multichannel intraluminal impedance (MII) measures electrical potential differences. A reflux episode is defined as a drop of the baseline impedance with >50%. The same criticism can be formulated as for pH monitoring: the clinical meaning of a drop with 49% or 51% is likely to be similar. Important to know also is that baseline is age dependent [154]. Moreover, esophagitis lowers the baseline; as a consequence, severe esophagitis might have a normalizing effect on MII results since baseline might be so low that a drop with >50% becomes unlikely. Interestingly, pH-only episodes, reflux episodes detected with pH metry but not with impedance (drop in pH without bolus movement) occur in young children [155]. While pH monitoring measures chemical clearance, MII measures bolus clearance. Therefore, reflux episodes last longer with pH monitoring than with MII. The detection of reflux by MII is not pH dependent, but in combination with pH metry, it allows detection of acid (pH < 4.0) and nonacid reflux, which can be divided further in weakly acid (pH 4.0–7.0) and alkaline reflux (pH > 7.0). Experience has shown that impedance needs to be performed in combination with pH metry, since pH-only events occur, mainly during the night and mainly in young infants. Also gas reflux is detected with MII, since liquid reflux causes a drop in impedance and gas reflux an increase. Interpretation of the recording is laborious and necessitates sufficient experience, since the automatic analysis is not standardized for children. Nevertheless, automated analysis seemed to be at least as reliable as analysis by experts [156]. Impedance seems especially of interest in patients with symptoms suggesting reflux but in the absence of esophagitis. Obviously, "more" reflux episodes are detected with MII-pH metry than with pH metry alone. The major clinical interest of MII seems to be demonstration of symptom association. Symptom Index, the percentage of reflux-related symptom episodes during a study period, does not incorporate the total number of reflux events in its calculation. Symptom association probability takes this limitation into consideration. But both parameters allow very well to interpret MII-pH as well off as on antiacid medication; the latter being of interest to differentiate between persisting (acid or nonacid) or absence of reflux in subjects with reflux symptoms refractory to clinical treatment. Debate regarding clinical normal data and validation of symptom association parameters in children are missing. The value of new metrics such as mean nocturnal baseline impedance of post-reflux swallow-induced peristal-

tic in children remains to be verified [137]. Also, the existence and implication of reflux hypersensibility (positive symptom association in the absence of pathologic reflux) and functional heartburn in children remains to be proven. Given the high cost of equipment and electrodes, the time needed for analysis and interpretation and the pros and cons in comparison to pH metry are debated.

Spectrophotometric esophageal probes to detect bilirubin are no longer used in children or adults and have been replaced by MII. Orel and coworkers showed that some children with esophagitis suffer from bile reflux [157].

Indirect techniques have been developed, mainly to diagnose GER(D) in patients with extra-esophageal manifestations. Accumulation of evidence regarding the determination of lipid-laden macrophages in bronchoalveolar liquid resulted in the conclusion that this method lacks sensitivity and specificity [1, 2]. More recent data show the presence of pepsin in bronchoalveolar lavage and middle ear fluid [1, 96]. Also bile salts are detected in middle ear fluid [158]. However, epidemiological data suggest a "protective" role of middle ear infection for the prevalence of GERD [1]. There is no data that acid-blocking medication has any effect on an ENT outcome as primary endpoint.

All GER investigation techniques test different aspects of reflux. Therefore, it is not unexpected that the correlation between the results of the different techniques is poor. There is no "always-best" investigation technique to diagnose GERD. "Logic interpretation" (but not evidence-based medicine) suggests that if the question asked is: "does this patient has esophagitis?", that endoscopy with biopsy is the best technique. If it is in the interest of the patient to measure acid GER episodes, 24-h pH metry is the preferred technique. But if quantification of all GER episodes is desired, MII-pH is likely to be the preferred technique. However, postprandial reflux is mainly weakly acid or alkaline, and postprandial reflux was considered to be not really relevant. Therefore, techniques measuring postprandial reflux (barium swallow, ultrasound, scintiscanning) are not recommended. Since therapeutic options are mainly limited to acid-reducing medication, it can be of interest to know the pH of the majority of the reflux episodes.

Therapeutic Options

Physiologic GER and regurgitation do not require medical drug treatment although they frequently cause parental distress and anxiousness. Any therapeutic intervention should strike a balance between intended improvement of symptoms and risk for adverse effects. Therapeutic options vary from reassurance, nutritional and positional treatment, prokinetics, and acid-reducing medications to surgery (Table 10.5).

Table 10.5 Schematic therapeutic approach in 2020

Phase 1	Parental reassurance Observation. Lifestyle changes. Exclude overfeeding
Phase 2	Dietary treatment (to decrease regurgitation and infant distress)
	Thickened formula, thickening agents, (thickened) extensive hydrolysates, or amino acid-based formula in cow's milk allergy
	Positional treatment (°)
Phase 3	For immediate symptom relief: Alginates (some efficacy in moderate GERD); antacids only in older children
Phase 4	Acid secretion blocking medication in ERD (proton-pump inhibitors are drug of choice; more safety data needed)
Phase 5	Prokinetics in NERD (theoretical concept, but no drug has been shown to be effective)
Phase 6	Laparoscopic surgery. Consider baclofen before surgery

Adapted from Vandenplas et al. [1]

Legend. Efficacy and safety data in infants and children for most anti-GER medication are limited

(°) limited data on 40-degree supine sleeping position in infants, *(N)ERD* (non) erosive reflux disease, *GERD* gastroesophageal reflux disease

Complications of Non-intervention

Although reviews on the natural evolution of regurgitation are available [8, 49, 50], there are only limited data on the natural history of GERD in infants and children because most patients do receive treatment at some point.

Traditionally, the impact of regurgitation on the long-term quality of life is trivialized since regurgitation is transitory in the vast majority of infants. However, recent data suggest a decreased quality of life in a number of parents of infants presenting with frequent regurgitation, even if the regurgitation has disappeared [80]. Infants spilling during 90 days or more during the first 2 years of life are at a greater risk for GER symptoms at 9 years [50]. Frequent versus infrequent infant spitting is related to a relative risk at 9 years of 2.3 (95% CI 1.3 – 4.0) for at least one GER symptom, of 4.6 (95% CI 1.5 – 13.8) for heartburn, of 2.7 (95% CI 1.4 – 5.5) for vomiting, and of 4.7 (95% CI 1.6 – 14.0) for acid regurgitation [50, 51]. It is unclear if regurgitation is less frequent in breastfed than in formula-fed infants, since data are contradictory [8, 50]. Gender is not a confounding factor, but smoking is [24]. Although symptoms improved in more than half of the infants with reflux esophagitis followed longitudinally for 1 year without pharmacotherapy, histology

remained abnormal in all [159]. But it is not known if treatment of regurgitation, GER, and GERD during infancy changes the outcome in adults.

In Conclusion: Although there is consensus that regurgitation and physiologic GER do not need medical drug treatment, some data suggest that nonintervention may have a negative impact on the quality of life of the child and the family.

Non-pharmacological and Non-surgical Therapies for GER

The most common reason for parents to seek medical help for young infants is frequent troublesome regurgitation and infant distress. Infants with troublesome regurgitation are difficult to distinguish from infants with mild to moderate GERD symptoms. The difference between "physiologic" and "pathologic" GER should not be regarded as a clear delineation, but as a continuum where "at some point" "normality" stops and "disease" begins. Parental coping plays a major factor in this process. Nonpharmacologic treatment such as reassurance, dietary, and positional treatment is recommended as an appropriate first approach in this group.

Reassurance while showing compassion for the impaired quality of life is of importance [2, 160, 161]. Keeping the infant for half an hour or so upright after feeding will also be helpful. In many situations, reassurance means observation of feeding and recommendations regarding appropriate handling of the child during and after feeding (Fig. 10.4: practical algorithm for the management of infant regurgitation). In formula-fed infants, the volume offered per feed should be appropriate according to the age and weight of the child [2, 160]. However, there are no data that relate ingested volume to frequency and volume of regurgitation, although it seems logic to hypothesize that feeding of large volumes favors regurgitations since it will increase TLESRs.

Similarly as for infant crying, parental report during a first consultation may overestimate the incidence of regurgitation [161]. These findings question the efficacy of "reassurance and guidance" which in most studies turns around 20%. The efficacy of "nonintervention" seems to bring the overestimation back to reality.

Excessive regurgitation in the absence of alarm signs such as failure to thrive is not a reason to stop or adapt breastfeeding. Anti-regurgitation (AR) formula decreases the number of episodes of regurgitation and increases the number of infants that do not regurgitate [162]. AR formula meets up to its expectations as it decreases visual regurgitation, but it does not systematically decrease acid reflux according to pH monitoring data [163]. AR formula is "anti-regurgitation" formula and not an "antireflux" formula. Commercialized thickened formula is preferred to thickening agents added to formula at home because the nutritional content of the thickening agent and its effect on osmolarity has been taken in account in the global composition of the commercialized formula [2, 160].

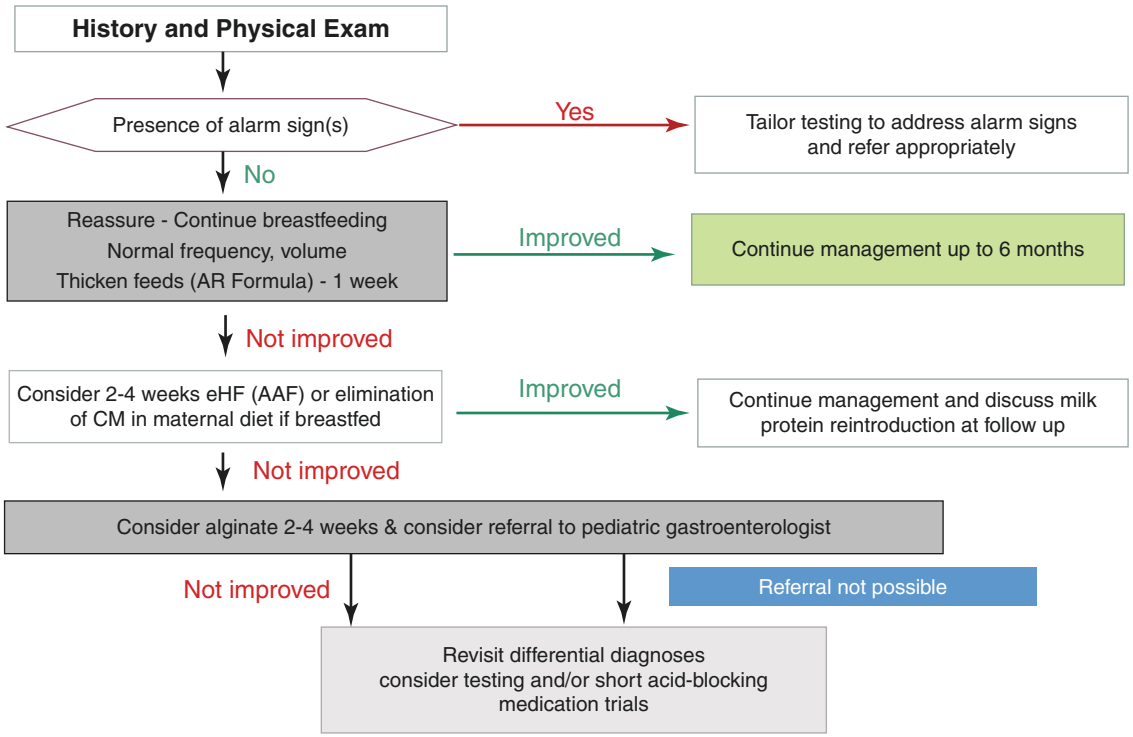
Bean gum is a popular thickening agent. Nutritional safety concerns have been raised based on the findings from an in vitro model that bean gum may hamper the absorption of micronutrients [164, 165]. However, in vivo data have contradicted this fear [166, 167].

Therefore, NASPGHAN and ESPGHAN recommend the use of AR formula, irrespective of the thickening agent (A1). Efficacy may be improved by changing the protein source to a partial or extensive hydrolysate [1, 163]. Partial hydrolysates may be more effective because of a better digestibility than intact protein and an enhanced gastric emptying [168]. CMPA may cause reflux, regurgitation, and vomiting and is often accompanied by a distressed behavior [1, 56]. Whether the efficacy of hydrolysates on reducing regurgitation, vomiting, and GER should be regarded as a proof of (non-IgE mediated) CMPA or because of an enhanced gastric emptying is not clear. The fact that postprandial weak acid reflux is increased after a cow milk challenge in children with CMPA [34] does not differentiate the pathophysiologic mechanism. A thickened extensive hydrolysate manages both conditions, and as a consequence does not differentiate between GERD and allergy [169].

Limited data suggest that probiotics, especially *Lactobacillus reuteri* DSM 17938 enhances gastric emptying and therefore decreases regurgitation [170, 171]. The same probiotic was shown to reduce regurgitation in breastfed infants [172].

Sleeping positions that have been suggested to reduce GER include prone, immediate right side with later left side after feeding, and supine 40° anti-Trendelenburg [173, 174]. Prone sleeping position is not recommended in infants because of the increased risk for sudden infant death. Van Wijk et al. concluded that the biggest benefit was achieved with a strategy of right lateral positioning for the first postprandial hour with a position change to the left thereafter to promote gastric emptying and reduce liquid GER in the late postprandial period [174]. However, independent studies reported a significantly increased risk of sudden infant death in side compared to the supine sleeping position [175, 176]. The results of a pilot study with the "Multicare AR-Bed®" suggest that this bed that nurses the infant in a 40-degree supine body position reduces regurgitation, acid reflux measured with pH monitoring, and reflux-associated symptoms evaluated with the I-GERQ (Fig. 10.5) [173].

In Conclusion: For noncomplicated reflux, management with medication is not recommended. Parental reassurance



AR: anti-regurgitation
eHF: extensively hydrolysed formula
AAF: amino acid formula
CM: cow's milk

18-9-2020

Fig. 10.4 Algorithm for the management of infant regurgitation

Fig. 10.5 The Multicare AR-Bed

and education regarding regurgitation and lifestyle changes are recommended. Positional treatment by keeping the infant upright for half an hour decreases regurgitation. Excessive regurgitation is not a reason to stop breastfeeding. Frequency and volume of formula should be adapted to the infant's weight and age. Thickened AR formula is effective in reducing regurgitation [1, 2, 177]. Data regarding probiotics are too limited to recommend routine administration. Hydrolyzed protein may offer an additional benefit.

Pharmacological Therapies for GERD

Pharmacotherapeutic agents used to treat GERD encompass antisecretory agents, antacids, surface barrier agents, and prokinetics. Pharmacologic treatment of infants with reflux symptoms is problematic as many infants have GER and little correlation is noted between reported symptoms and endoscopic or pH findings.

Prokinetics and Other Non acid-Reducing/Blocking Medication

From the pathophysiologic point of view, prokinetics are the most logic therapeutic approach to treat NERD in infants, since acid reflux is of minor importance in this age group. However, there is no effective and safe prokinetic agent on the market. Cisapride was probably the prokinetic drug for which efficacy data were strongest, although only a decrease of the reflux index (% time pH was <4.0) has been shown.

Cisapride significantly reduced the reflux index (weighted mean difference –6.49; 95% CI –10.13 to –2.85; $P = 0.0005$) [178]. But cisapride was taken off the market in the beginning of this century by the American and European authorities because of its cardiac adverse effects such as QT prolongation and torsades de pointes.

Other prokinetics are metoclopramide and domperidone. The efficacy studies with metoclopramide are limited and outdated [179, 180]. The seven metoclopramide studies performed in children under the age of 2 years are all from before 1990 and used a variety of outcomes [179, 180]. Compared to placebo, metoclopramide appears to reduce daily symptoms (SMD –0.73; 95% CI –1.16 to –0.30), and to reduce the reflux index (WMD –2.80%; 95% CI –5.58 to –0.01) [179]. Metoclopramide has a high incidence of adverse effects such as lethargy, irritability, gynecomastia, galactorrhea, and extra-pyramidal reactions and has caused permanent tardive dyskinesia. The most commonly reported adverse effects associated with the use of metoclopramide in children—extra-pyramidal syndrome, diarrhea, and sedation—were reversible and of no long-term significance [181]. No guideline recommends metoclopramide to treat GERD [1, 2, 180].

A systematic review of studies on domperidone identified only four randomized controlled trials in children, none providing evidence for efficacy [182]. Domperidone occasionally causes extra-pyramidal central nervous system side effects [65]. Domperidone-treated infants infrequently (i.e., <5%) displayed QTc prolongation at doses used for the management of GORD in infants [183]. Pathological QTc intervals were noted among a small number of infants, which supports the possibility of domperidone-associated risk of prolonged QTc interval [184].

Nevertheless, the lack of efficacy and the cardiac effects of domperidone did result in withdrawal of domperidone for infants [185–187]. As a consequence, domperidone is not recommended in GERD management [188]. Since 2019, the use of domperidone in newborns, infants, children (under 12), and adolescents weighing less than 35 kg is no longer approved at the European level. Other prokinetic molecules such as mosapride, itopride, prucalopride, and renzapride have not or insufficiently been studied in children.

In adults, metoclopramide and domperidon neither are recommended for the treatment of GERD [189]. Nevertheless, other prokinetics have been studied for their potential benefit in GERD patients, albeit in small studies. Itopride decreased both symptoms as well as pathologic reflux [190]. Recently, the combination of acotiamide with PPI demonstrated a significant improvement of symptoms as well as reflux parameters over PPI alone [191]. Combining mosapride with PPI was, on the other hand, not more effective than PPI alone in GERD [192–194].

Bethanechol, a direct cholinergic agonist, was studied in a few controlled trials and has uncertain efficacy and a high incidence of adverse effects [1, 2]. Baclofen is a gamma-aminobutyric acid (GABA)-B receptor agonist used to reduce spasticity in neurologically impaired patients. Baclofen was shown to reduce the number of TLSERs and acid GER during a 2-h test period and to accelerate gastric emptying [195]. Baclofen can be used as supplemental therapy to proton-pump inhibitors in children with refractory GER, although failure rate is around 33% [196]. The data on baclofen are very limited, and the high incidence of adverse events does not justify its widespread use. Also abaclofen did not fulfill its promises. However, experience is different in adults. In adults, add-on therapy with baclofen validated its ability to reduce GERD symptoms as well as to reduce acid exposure [197, 198], even in the presence of a hiatal hernia [199]. M0003 is a specific and high-affinity 5-HT4 agonist for the treatment of upper GI disorders, focusing initially on severe gastroparesis and pediatric reflux. Clinical studies have been postponed.

Erythromycin was mostly studied in infants with feeding problems and has been very poorly studied in GER [200]. Erythromycin was shown to not reduce GER in preterm infants [201]. Moreover, prolonged use is associated with loss of efficacy because of tachyphylaxis. In adults, azithromycin was shown to reduce acid reflux in patients with a small hiatal hernia and to reduce reflux after lung transplant [202, 203].

In conclusion Although the prokinetic concept is of interest, there is no effective prokinetic on the market. All prokinetics have a high incidence of adverse effects. The North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) and European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) practice guidelines concluded that there is insufficient evidence to justify the routine use of prokinetic agents [1, 2].

Alginate(-Antacids) and Mucosal Protectans

Alginate(-antacids) have mainly been validated in adults. Their key therapeutic advantage is their rapid onset of action, within minutes from ingestion. Results showed a marginal but significant difference between Gaviscon Infant and placebo in average reflux height being better for placebo [204]. Although the NASPGHAN/ESPGHAN guidelines did not recommend alginates because of lack of evidence [2], the NICE guidelines and a Cochrane review concluded that the evidence was sufficient to recommend a 2-week trial [205, 206]. An open trial showed improvement of clinical symptoms but also of impedance parameters during 24-h administration of alginate [207].

There is more experience with mucosal protectans in adults than in children. In adults, some studies indicate adequate symptom relief and improvement of quality of life with the combination of chondroitin sulfate and hyaluronic acid in a bioadhesive formulation alone or when combined with PPI [208, 209]. However, as data in children are lacking, mucosaprotectants so far have no role in the management of GERD in children [1, 2].

In conclusion: Based on expert opinion, there may be a transitory place for alginates in patients with GERD in whom rapid symptom resolution is required, but there is no evidence for this recommendation. The value of hyaluronic acid and chondroitin sulfate in children remains unknown and should therefore not be recommended as yet.

H^2-Receptor Antagonists (H_2RAs) and Proton-Pump Inhibitors (PPIs)

Ranitidine is by far the most popular H_2RA. Since PPIs are more effective to suppress acid than H_2RAs, PPIs are considered the preferred option for the treatment of acid GERD in children and adults [210]. Some infants presenting symptoms assumed to be caused by acid GER fail to respond to acid suppression [211]. It has been shown in adults and children that PPIs do not reduce the incidence of reflux episodes [212, 213]; they change the pH of the refluxate from acid to nonacid or weakly acid.

Because PPIs in liquid formulation are only available in a very limited number of countries, H_2RA sirup remains popular since it is available worldwide, making it easier to administer in infants and young children. However, ranitdine has been withdrawn from the market in many countries because of the presence of nitrosamines, a carcinogen [214]. The sirup does also contain minimal amounts of alcohol. The prescription rates of antiacid medications are still on the increase [215]. Between 2002 and 2009, there was an 11-fold increase of the use of PPI in the USA [2]. In 2010, lansoprazole was the 9th and ranitidine the 14th most prescribed drug in children under 2 years of age in the USA [74].

Since gastrin release after a meal is one of the most potent activators of H^+–K^+-ATPase, PPI should be administered long enough before a meal to be absorbed, but not eliminated, by the time the proton pump is activated [216]. It may be preferred to administer PPI once a day, before breakfast because young children eat frequently during the day. PPI must be protected from gastric acid by enteric coating; the granules and tablets should not be crushed, chewed, or dissolved as gastric acid secretion may alter the drug. If the microgranules are enteric coated, the capsules can be opened and administered orally or via a feeding tube, in suspension in an acidic medium such as fruit juice, yogurt, or apple sauce. A "home-made" liquid formulation, produced by dissolving the granula, not the microgranula, in 8.4% bicarbonate solution has been developed [1, 2].

Omeprazole is approved in the USA and Europe for use in children older than 1 year of age; in the USA, lansoprazole is approved as well. Esomeprazole is approved in the USA for short-term treatment of GERD with erosive esophagitis in infants aged from 1 to 12 months [177]. In Europe, approval for esomeprazole is identical to the approval of omeprazole. Pharmacodynamics and -kinetics have been studied for many PPI molecules in different age groups. In children aged 6 to 16 years with GERD, currently available pantoprazole delayed-release tablets can be used to provide systemic exposure similar to that in adults [217]. Recommended dosages of antiacid medications are listed in Table 10.6. When PPI need to be administered through a nasogastric tube, the most satisfactory results were obtained with lansoprazole orally disintegrating tablet [218]. A 5 ml volume of water diluent for suspension and a 10 ml volume of flush-through water made it possible to deliver the full lansoprazole dose [219].

Authorities seem to approve PPIs in neonates, infants, and children rather on pharmacologic studies than on effectiveness in the treatment of GERD [216, 219]. Rectal administration of PPI has scarcely been investigated; there are no data comparing its efficacy with oral intake.

Table 10.6 Drugs and recommended dosages for the treatment of GERD

Drug (commercial name)[a]	Recommended dosage	Max dosage (adults)
Prokinetics		
Metoclopramide (Primperan®)	0.4–0.9 mg/kg/day	60 mg
Domperidone (Motilium®)	0.80–0.9 mg/kg/day	30 mg
Baclofen (Lioresal®)	0.5 mg/kg/day	80 mg
Antacids		
Sodium alginate (Gaviscon®)		
Histamine-2-receptor antagonist (H2RA)		
Ranitidine (Zantac®)	5–10 mg/kg/day	300 mg
Nizatidine (Axid®)	10–20 mg/kg/day	300 mg
Famotidine (Pepcidin®)	1 mg/kg/day	40 mg
Proton-pump inhibitors (PPIs)		
Omeprazole (Losec®)	1–4 mg/kg/day	40 mg
Esomeprazole (Nexium®)	10 mg/day (weight <20 kg)	
	20 mg/day (weight >20 kg)	40 mg
Lansoprazole (Prezal®, Prevacid®)	2 mg/kg (infants)	30 mg
Panotoprazole (Pantomed®)	1–2 mg/kg/day	40 mg
Dexlansoprazole (Dexilant®)	>12 years	60 mg

[a]All these drugs can be prescribed under pharmacologic name (except Dexlansoprazole)

The belief that many infants do present with (excessive) crying because of (excessive) esophageal acid exposure has become extremely popular over the past 10 years. Many infants have periods of unsettledness, irritability, and distress during the first months of life. Spilling due to reflux of gastric contents is also seen very frequently. These are in general part of the normal patterns of infancy that resolve with time. In recent years, these normal developmental processes have increasingly been ascribed to pathology and treated with medical therapies, including acid suppressants [220]. GERD may cause excessive crying in infants [52]. The concept that infant irritability and sleep disturbances are manifestations of GER is largely extrapolated from adult descriptions of heartburn and sleep disturbances that improve with antacid therapy [1, 2, 221]. However, there is no evidence to subscribe to this hypothesis in children. In the study by Heine et al., all infants with pathological GER presented with frequent vomiting, and "silent" pathological reflux did not occur, indicating that pathological GER is an unlikely cause of infant irritability under the age of 3 months [52]. Several placebo-controlled prospective trials with PPIs in infants presenting with "excessive crying and reflux-like symptoms" have been performed and show negative results, with an increased incidence of adverse events in the PPI group sometimes as being the only difference in outcome [81, 217, 219]. Antiacid medications are among the most commonly prescribed medications in neonatal intensive care units in the USA because they are administered to treat clinical signs thought to be caused by GER, such as apnea, bradycardia, or feeding intolerance, despite the lack of evidence of efficacy in this population [74, 222]. Use of antireflux medications at the time of discharge seems to be common for extremely low birth weight infants [65]. The lack of evidence for a benefit of PPIs in neonates seems not to determine the beliefs of physicians [223]. Prolonged treatment of pediatric patients with PPIs has not caused cancer or significant abnormalities [219]. However, many adverse effects related to PPI use have been reported in both children and adults, such as small bowel intestinal overgrowth, idiosyncratic reactions, drug-drug interactions, hypergastrinemia, hypochlorhydria, and many others [1, 2, 215]. Idiosyncratic reactions occur in up to 12–14% of children taking PPIs: headache, diarrhea, constipation, nausea [1, 2]. Acid suppression or hypochlorhydria causes an abnormal gastrointestinal microbiome and bacterial overgrowth in up to 30% of patients [224]. As a consequence, the prevalence of infectious respiratory and gastrointestinal tract infections is increased [215]. PPIs are associated with an increased risk of colonization and infection with *Clostridium difficile*. PPIs, particularly if administered for <30 days or in a high dose, showed an association with community-acquired pneumonia [215]. Acute interstitial nephritis has repetitively been attributed to PPI [225]. Hypomagnesemia is reported as a rare but severe complication. PPI are reported to be associated with an impairment of bone mineralization [215]. Gastric acid suppression predisposes patients to develop allergy [215, 226]. Antiacid medication during pregnancy was reported to increase the risk to develop asthma in the offspring [215]. In adults, retrospective studies detected an association of long-term PPI use and dementia, further questioning its widespread use in the youngest [227, 228].

Where PPI definitively inactivate the H^+–K^+-ATPase, potassium-competitive acid blockers reversibly inhibit it. The long-term efficacy of vonoprazan in GERD has been demonstrated in adults [229]. However, according to a meta-analysis, this effect was not superior to PPI in general, with the possible exception of rabeprazol [230, 231]. No studies have studied its potential in children.

In adults, awareness of possible side effects of inappropriate PPI treatment initiated research on deprescribing PPI [232]. During this process, some patients will experience rebound acid hypersecretion as a result of PPI-induced hypergastrinemia. This results in symptom recurrence, hampering cessation of treatment. It is therefore mandatory to warn the patient of this possibility. Different approaches are possible: on-demand treatment, dose reduction, and abrupt cessation of PPI. In 2017, a meta-analysis conducted by the Cochrane collaboration indicated that on-demand treatment resulted in a reduction of pill burden, with possible associated increased in GI symptoms [233]. Based on results from different studies, dose reduction is possible in 50–88% of GERD patients [232]. There are insufficient data to favor one approach above the other. Guidelines for deprescribing PPI in adults are available [234, 235]. Both guidelines focus on the need to limit the duration of PPI treatment and emphasize the importance of lifestyle measures, as well as the use of alternative medication like H2RA of antacids. Stopping of PPI is a matter of debate in pediatrics. Most centers arbitrarily decrease the dosage by 50% during 1 week. However, since there are no studies, there is absence of evidence.

In conclusion: If acid-reducing medication is indicated, PPIs are the best choice. PPIs are effective in healing reflux esophagitis in children of all ages but do not improve GER-related symptoms in infants. PPIs are overused in newborns, infants, and young children. Adverse effects of PPIs should be considered, as they are frequent, and the drug is often prescribed when not indicated. Knowledge on the best approach to stop long-term inappropriate PPI treatment is lacking.

Surgery and Therapeutic Endoscopic Procedures

Different antireflux surgical approaches do exist. In general, experience seems to be the best guidance for choosing the technique. Antireflux procedures in the USA were

reported to be most commonly performed in children during a period of life when regurgitation is normal and physiologic and objective measures of GERD are difficult to interpret [236]. In contrast, in Europe, antireflux surgery is considered as a treatment of last resort in children with GERD refractory to pharmacological therapies [237]. Recent data suggest that the selection of patients who will benefit from surgery might be enhanced by automated impedance manometry pressure-flow analysis, which relates bolus movement and pressure generation within the esophageal lumen [237]. In adults, most benefit from antireflux surgery is observed in patients with typical reflux symptoms well controlled with PPI and patients with demonstrated pathologic esophageal acid exposure [238]. The management of antireflux surgery in PPI-refractory GERD is mainly based on expert opinion [239].

Fundoplication in children reduces GER without altering esophageal motility [240]. Primary fundoplication provided control of symptoms in almost 90% of patients and also reduced the rate of esophagitis. Failure of primary fundoplication occurred in 15% of patients, and an underlying disorder, esophageal atresia, and hiatoplasty increased the risk of failure [241]. The LOTUS study prospectively evaluated the efficacy of omeprazole versus antireflux surgery in GERD adult patients over a 5-year time interval. After controlling for study dropout, no difference in remission rate was identified between both groups [242]. Nevertheless, prior adult series report that between 37% and 62% of patients are again on PPIs a few years after surgery [243, 244]. Since laparoscopic antireflux surgery is much less invasive than open surgery, causing less operative pain, faster recovery, shorter hospital stay, and a better cosmetic result, only laparoscopic surgery should be performed. Long-term outcome of open and laparoscopic interventions is similar. While antireflux surgery in certain groups of children may be of considerable benefit, a failure rate of up to 22% has been reported [1]. Long-term follow-up after laparoscopic fundoplication produces a good clinical result and a good quality of life [245]. Surgery for GER patients have a significant improvement in their quality of life, not only by the reduction of their symptoms but also in enhancing from the nutritional status [246]. Efficacy of antireflux surgery in neurologically impaired children may be similar to normally developed children [247].

The outcome of antireflux surgery does not seem to be influenced by different surgical techniques, although postoperative dysphagia may occur less after partial fundoplication [247]. Laparoscopic fundoplication is a safe procedure in infants ≤5 kg without increase of postoperative complications, recurrence, or mean operative time [248]. Failure of Nissen fundoplication is particularly frequent in patients previously operated upon for esophageal atresia or congenital diaphragmatic hernia [249]. A redo Nissen is indicated if symptoms of GER recur, but the proportion of failure of a redo is even higher [249].

Table 10.7 Studies evaluating the efficacy of PPI in newborns, infants, and children (updated 2019)

Author (year)	Paper (Ref)	Age	Results
Gold BD (2007)	OA [256]	12–17 years	Symptom improvement
Gilger MA (2008)	OA [257]	1–11 years	Symptom improvement
Baker R (2010)	OA [258]	1–5 year	Pantoprazole effective in GERD with esophageal symptoms
Higginbotham TW (2010)	Rev [259]	Infants and children	PPIs are not effective in common infant GERD-associated symptoms
Tolia V (2010)	OA [260]	1–11 years	Esomepazole heals macroscopic and microscopic erosive esophagitis
Winter H (2010)	OA [261]	1–11 month	No significant differences between pantoprazole and placebo in withdrawal rates due to lack of efficacy
Van der Pol R (2011)	Rev [262]	Infants	PPIs not effective in reducing GERD symptoms
Lee JH (2011)	OA [263]	4–18 years	PPIs are effective
Winter H (2012)	(OA) [264]	1–11 month	No significant differences between esomeprazole and placebo in withdrawal rates due to lack of efficacy
Ummarino D (2012)	OA [265]	1–181month	PPI > H^2RA for respiratory symptoms
Davidson G (2013)	OA [266]	Neonates	PPIs are not effective
Tjon JA (2013)	Rev [267]	0–16 years	RCTs and systematic reviews: lack of efficacy of PPIs, specifically in young infants
Shashidhar H (2013)	Rev [268]	0–18 years	PPI are effective in healing reflux esophagitis in children of all ages but do not improve GER-related symptoms in infants
Tighe M (2014)	Rev [205]	0–16 years	Moderate evidence was found to support the use of PPIs, along with some evidence to support the use of H_2 antagonists in older children with GERD PPI in infants with functional GER have demonstrated variable benefit
Gold BD (2017)	OA [269]	12–18 years	PPI are effective in NERD in adolescents
Gremse D (2019)	OA [270]	12–18 years	PPI are effective in erosive esophagitis in adolescents
Shimizu T (2019)	OA [271]	1–14 years	PPi are effective in gastric acid-related disease

OA original article, *rev* review

The table is not exhaustive but lists the papers on PubMed between 2007 and 2019 with "proton-pump inhibitor infant" and "proton-pump inhibitor pediatric" as search terms and illustrates the differences in outcome according to age of the patients

Children with underlying conditions predisposing to the most severe GERD comprise a large percentage of many surgical series. The robot-assisted Nissen fundoplication in children is a safe alternative to conventional laparoscopic surgery [250]. Cardiaplication results in an increase in cardia yield pressure in young pigs. This procedure may be an alternative antireflux operation for infants [251].

Therapeutic endoscopic procedures are rarely indicated and should only be performed in units where there is experience [252]. Different endoscopic techniques have been developed over the years, but only the transoral incisionless fundoplication procedure and radiofrequency application survived the test of time [253]. The transoral incisionless fundoplication procedure can complement the current surgically and medically available options for children with GERD, especially in complicated patients such as those with neurological impairment [254]. In a small series, radiofrequency application resulted in complete symptom remission in 5 of 6 children with recurrent GERD after previous antireflux surgery [255]. Total esophagogastric dissociation is an operative procedure that is useful in selected children with neurologic impairment or other conditions causing life-threatening aspiration during oral feedings (Table 10.7).

In conclusion: Surgery in indicated when symptoms are life-threatening or when a child beyond the age of 2–3 years is depending on chronic treatment with antiacid medications. In neurologically impaired children, the risk and benefit of a surgical intervention should be well balanced. Nissen fundoplication in neonates and young infants should only be reserved to selected infants failing medical therapy and suffering from life-threatening complications of GERD [177]. Local experience with a given surgical procedure seems the best outcome predictor.

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Esophageal Achalasia

11

Efstratios Saliakellis, Anna Rybak, and Osvaldo Borrelli

Introduction

Achalasia literally denotes the inability to relax (from the Greek words α—"not," χάλασις—"relaxation"). Sir Thomas Willis first described the classical symptomatology of achalasia in a patient with dysphagia to liquid food in 1672 [1]. He treated this individual by dilating the esophagus using a sponge attached to the tip of a whalebone. The term "achalasia" was first introduced in 1924 by Hurst in an attempt to describe the inability of the lower esophageal sphincter (LES) to relax in affected individuals [2]. Despite the lack of a clear explanation of the etiopathogenesis of the disease, an effective surgical treatment was proposed by Heller in 1913 [3].

Esophageal achalasia is a primary motility disorder of the esophagus. It is clinically characterized by various degrees of dysphagia to solids and liquids in the absence of other conditions that could possibly cause the same clinical symptoms (e.g., esophageal stricture, eosinophilic esophagitis). The radiological cardinal features consist of abnormal esophageal peristalsis, dilation of the esophageal body, and "bird-beak" appearance at the level of the LES with concomitant delayed emptying of the contrast material from the esophagus. Endoscopically, this condition is generally characterized by esophageal dilation, presence of saliva or undigested food within the esophagus, and resistance in passing the endoscope through the LES. Manometrically, this clinical entity is defined by the presence of variable impairment of esophageal peristalsis and inability of the LES to relax following swallowing [4]. Primary or idiopathic achalasia is the appropriate term to be used when there is no clear etiology for the disease, whereas in secondary achalasia the cause is by definition known [5].

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Epidemiology

There are relatively limited data regarding the incidence of esophageal achalasia in children. It is, however, a rare disease [6], and according to three more recent studies its incidence in the pediatric population has been estimated between 0.1 and 0.3 cases annually per 100,000 children [7–9]. This clinical entity can be encountered at any age; however, it seldom occurs in infancy as only 6% of the diagnoses have been made in this age group [10]. The peak incidence of the disease occurs between 30 and 60 years of age [11, 12]. Although there are data in adult population revealing equal gender distribution, childhood achalasia is more frequent in males than females [10]. Available data do not support any racial predilection among affected individuals [4].

Heredity

The majority of patients with achalasia are sporadic cases. Familial achalasia is less frequent [10] and is more commonly encountered among monozygotic twins and offspring of consanguineous parents. The latter may be suggestive of an autosomal recessive inheritance [13–15].

Etiopathogenesis

Despite the fact that achalasia was described more than 300 years ago, its etiopathogenesis remains largely enigmatic [16]. This condition is likely to be multifactorial in origin [5]. It has been postulated that host genetic factors, autoimmunity, and environmental influences contribute to the initiation of an inflammatory neurodegenerative process. The end result is a reduction in the number of the ganglion cells in the myenteric plexus of both esophageal body and LES [17]. During the initial stage of the disease, the neurodegenerative process affects the inhibitory neurons and their

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neurotransmitter nitric oxide (NO) more than the excitatory neurons and neurotransmitters, such as acetylcholine, resulting in both high-amplitude non-propagative contractions of the esophageal body and non-relaxing LES, which is described by the term "vigorous achalasia" [17–20]. The disease gradually progresses, and cholinergic neurons are affected as well leading to "classical achalasia," which is characterized by very low-amplitude esophageal body contractions or aperistalsis in addition to an inability of the LES to relax in response to deglutition [21–24]. There are data suggesting that neurodegeneration might not be limited to the esophagus only as abnormalities (Wallerian degeneration) have been also demonstrated in the vagus nerve of affected humans and experimental animal models [25, 26]. Furthermore, abnormal findings in the brainstem (Lewy bodies), prolonged gastric emptying time, and disturbed response of gastric secretions to hypoglycemia have also been reported in achalasic patients [27–29].

Despite many advances in our understanding of achalasia [30], the triggers of neurodegeneration are poorly defined. Infections by various agents (e.g., measles, varicella-zoster virus, herpes simplex viruses type 1, 2, 6, human herpesvirus 6, Epstein–Barr virus, human papillomaviruses) have all been implicated in the pathogenetic process [31, 32]. However, the role of these viruses has been questioned by other authors [33, 34]. Thus, with the exception of Chagas disease caused by Trypanosoma cruzi, there is inadequate evidence to support a definitive causative association between infections and esophageal achalasia [35]. Autoimmunity has also been proposed as a key factor in the pathogenesis of esophageal achalasia. The latter was postulated by the identification of inflammatory cells [21, 36–38]. Moreover, the identification of specific human leukocyte antigen (HLA) class II histocompatibility antigens in combination with the presence of antimyenteric autoantibodies [39, 40] further strengthened the hypothesis of the autoimmunity involvement in the disease's pathophysiology. However, these findings have not been confirmed by subsequent studies [41, 42]. In conclusion, current evidence suggests that there may be an element of autoimmune predisposition to achalasia for certain individuals, but the available data do not support a definition of achalasia as an autoimmune disease.

Certain reports of achalasia in siblings, monozygotic twins along with familial cases [43–46] raised the possibility of a potential genetic hereditary influence. The latter is particularly true for the cases of achalasia in the context of Allgrove syndrome in which a correlation has been identified between the esophageal dysmotility and the ALADIN gene [47, 48]. The potential genetic basis of this clinical entity is further highlighted by the association of achalasia with other diseases with proven genetic influence (Trisomy 21, Parkinson disease, Rozycki syndrome) [49–51]. Moreover, specific polymorphisms in the genes of NO synthase (NOS), vasoactive peptide (VIPR1), interleukin-23 receptor (IL-23R), interleukin-10 (IL-10) promoter, and protein tyrosine phosphatase non-receptor 22 (PTPN22), along with abnormalities of guanylate cyclase (the major nitric oxide receptor) and disruptions in the nitric oxide signaling, may be associated with achalasia providing additional data regarding the pathophysiology of idiopathic achalasia. However, more studies are required to further elucidate the details of the downstream responses that ultimately impair the esophageal body motility and render the LES incapable of relaxation [52–58].

In contrast to idiopathic achalasia in which the cause remains largely unknown, in secondary achalasia, the causative factor can be identified. Table 11.1 depicts certain pathologies which are associated with achalasia-like motility disorders [59–72].

Table 11.1 Pathologies associated with achalasia-like esophageal dysmotility

Chagas disease
Tumors (e.g., leiomyoma, Hodgkin's disease, gastric carcinoma; "pseudoachalasia")
Sarcoidosis
Hereditary cerebellar ataxia
Eosinophilic esophagitis
Hirschsprung's disease
Chronic idiopathic intestinal pseudo-obstruction
Multiple endocrine neoplasia type 2b
Miscellaneous (e.g., juvenile Sjogren's syndrome, moyamoya disease, Ondine's curse, autism)

Clinical Presentation

The clinical picture of achalasia depends on the duration of symptoms and the age of the child at the time of the diagnosis [73]. It has been reported that childhood achalasia is more common in males and is largely occurring in school-age children (7–12 years). However, achalasia may occur during infancy [74]. Cases have also been reported in premature neonates as young as 29 weeks of gestation and as small as 900 g [75, 76]. The mean duration of symptoms prior to diagnosis was less than 3 years in 80% of the children, and the chief complaints are represented by dysphagia and emesis [10]. Table 11.2 summarizes the clinical symptoms of the children reported in the literature [77]. Noteworthy, children younger than 5 years of age present more frequently with vomiting, whereas dysphagia is the predominant complaint in older children [73]. Dysphagia is progressive and initially confined to solids, and in later stages to both solid and liquid. The child usually reports a sensation of food getting "stuck" in the esophagus ("chest") which is usually relieved by multiple swallowing efforts or by washing the food bolus down

Table 11.2 Chief complaints in childhood achalasia

Chief complaints in childhood achalasia	Frequency (%)
Regurgitation/vomiting	80
Dysphagia	76
Loss of body weight	61
Respiratory tract symptoms	44
Thoracic pain	38
Faltering growth	31
Regurgitation at nighttime	21

Table 11.3 Differential diagnosis of esophageal achalasia

Gastroesophageal reflux disease
Esophageal stricture
Eosinophilic esophagitis
Asthma
Tumors (pseudoachalasia)
Rumination syndrome
Eating disorders
Chagas disease
Miscellaneous

with liquids. The troublesome deglutition leads to limited oral intake (food refusal) and subsequently in suboptimal weight gain (failure to thrive) or weight loss. As the disease progresses, the esophagus becomes dilated and the patient may experience regurgitation of undigested food or saliva that accumulates at nighttime while the child is asleep, leading also to episodes of recurrent coughing and choking. The latter may predispose the child to a significant risk of chest infections or even sudden death due to aspiration of esophageal contents [78].

Diagnostic Approach

Diagnosis is often delayed due to several factors, including its low incidence and the inability of younger children to report the symptoms, which are often nonspecific. Moreover, most of the related symptoms and signs, such as emesis, respiratory involvement, and failure to thrive, are commonly attributed to gastroesophageal reflux (GER) disease. Therefore, a detailed history and a careful clinical examination are paramount as they can expedite the diagnostic process by guiding the physician in the correct choice of investigations which will eventually establish the diagnosis. Some individuals will have associated alacrima and adrenal insufficiency (AAA syndrome) and may have dermal hyperpigmentation secondary to high blood adrenocorticotropic hormone (ACTH) concentrations. Radiological, endoscopic, and manometric procedures represent the diagnostic arsenal and are the recommended modalities for the diagnosis of achalasia [4]. Esophageal achalasia needs to be distinguished from other conditions that present with regurgitation, vomiting, and dysphagia. Table 11.3 presents the entities that physicians should include in the differential diagnosis of esophageal achalasia [4, 67, 79–87].

Radiology

Upper gastrointestinal (GI) contrast series (esophagography/esophagogram/barium swallow) provides a convenient way to assess the anatomy of the upper GI tract. They are usually readily available and as a result can expedite the diagnostic assessment or confirm the diagnosis in many of the suspected cases of achalasia. In spite of its undisputed usefulness, reports from adult studies showed that esophagogram can be nondiagnostic in up to one-third of cases [88]. These data, however, were not substantiated in the pediatric population where 92% of the studies demonstrated abnormal findings [10].

The esophagogram usually reveals various degrees of esophageal body dysmotility with or without esophageal dilation, narrowing of the lumen at the level of esophagogastric junction (EGJ; "bird-beak" or "rat-tail" appearance), poor emptying of the contrast material into the stomach, and prominent tertiary contractions in the case of vigorous achalasia [10, 89–92] (Figs. 11.1, 11.2, and 11.3). Esophagography supports the diagnosis in the case of an equivocal manometry and can also demonstrate findings of end-stage achalasia (e.g., tortuosity and angulation of the esophageal body, megaesophagus) which may modify the clinical decision regarding the most appropriate therapeutic approach [4, 93–97]. Additionally, radiology can be used as an objective tool to evaluate the response to treatment (the so-called timed barium esophagogram (TBE), which measures the height of the barium column in the upright position after an ingestion of a large barium bolus) [98, 99], and as a predictor of treatment's efficacy [100–102].

Upper Gastrointestinal Endoscopy

Endoscopy has an important role in the diagnosis of achalasia as it rules out clinical entities that can mimic achalasia, such as eosinophilic esophagitis and other causes of pseudoachalasia. It can also alter the initial working diagnosis for patients erroneously treated for GER [4, 103, 104]. The major endoscopic findings raising a high index of suspicion for achalasia include esophageal dilation, presence of retained saliva or food along with resistance while negotiating the LES. Mucosal abnormalities detected during endoscopic examination may be due to food stasis and candida infections. However, it is not unusual to witness an unremarkable upper GI endoscopy in the early stages of the disease [4].

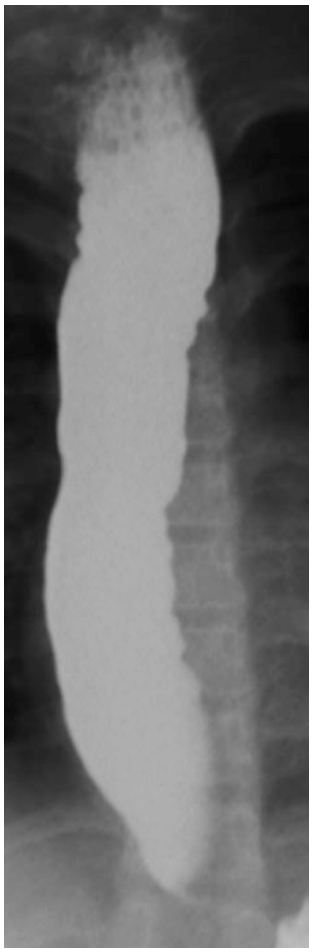

Fig. 11.1 Image of esophagus from barium esophagram in an 11-year-old female with achalasia. There is food residue in the upper esophagus. The esophagus is dilated and shows numerous irregular contraction waves (tertiary contractions)

Of significant note, an increased number of eosinophils have been reported in the esophageal biopsies of achalasia patients [105]. While the interplay among achalasia and esophageal eosinophilic infiltration needs to be further elucidated, it has been suggested that the latter does not represent a distinct clinical entity. Thus, the presence of esophageal eosinophils in patients complaining of dysphagia may warrant further diagnostic work-up to rule out other potential etiologies, including achalasia [106]. High-resolution manometry (HRM) is considered an invaluable tool for discriminating these two entities as they generally have distinctive motor patterns [107–109].

Except for its diagnostic role, upper gastrointestinal endoscopy has been proposed to be a useful intraoperative tool that can help in guiding the extent and adequacy of Heller's myotomy and also enables the operators to assess the integrity of the esophageal mucosa during the procedure [110].

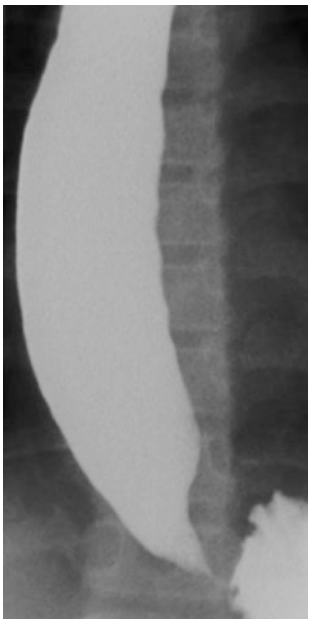

Fig. 11.2 Lower esophagus from barium esophagram in an 11-year-old female with achalasia. The lower esophagus is dilated; there is tapered ("beak-like") narrowing of the gastroesophageal junction

Manometry

Esophageal manometry provides a highly sensitive and specific method for defining the esophageal motility pattern [111]. This is especially true for the combined high-resolution esophageal impedance manometry [112]. Pediatric patients are assessed with a standardized protocol that involves single and multiple rapid wet swallows and solid swallows [109]. The esophageal motor abnormalities found in patients with achalasia are classified into three subtypes of achalasia according to recently introduced Chicago classification [113]. All three subtypes are characterized by elevated integrated relaxation pressure (IRP—a variable which quantifies residual LES pressure): type I is defined by completely failed esophageal body peristalsis (Fig. 11.3b), in type II there is abnormal peristalsis accompanied by pan-esophageal pressurization in more than 20% of the test swallows (Fig. 11.3c), and type III also demonstrates an impaired contractile pattern with fragmental preservation of distal peristalsis or the presence of premature (spastic) contractions in more than 20% of the swallows (Fig. 11.3d) [113]. Available data in pediatrics suggest that there is a high intra- and interrater variability in diagnosing esophageal achalasia along with its subtypes (Chicago classification) when using HRM as a diagnostic modality [114, 115]. The results of the "European achalasia trial" demonstrated that HRM may facilitate clinical decision regarding the most appropriate initial treatment according to the manometric subtype [116]; there are also

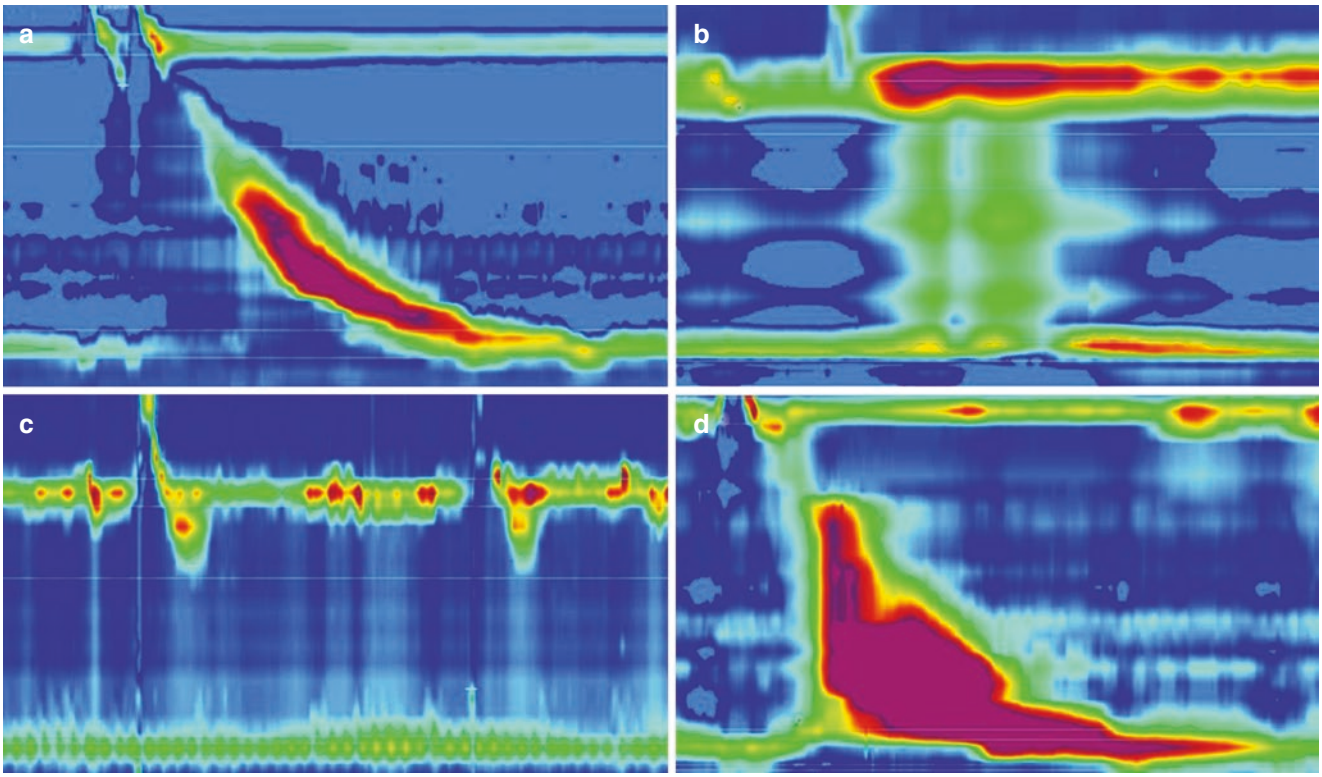

Fig. 11.3 High-resolution esophageal manometry in a healthy (**a**) and three achalasic children. Type I (**b**) is characterized by the absence of distal pressurization to greater than 30 mmHg. In type II (**c**), pressurization to greater than 30 mmHg occurs in at least two of ten test swallows, whereas patients with type III (**d**) disease have spastic contractions with or without periods of compartmentalized pressurization

data supporting the fact that preoperative HRM in children may help in predicting the response to surgery [117]. Furthermore, some data suggest that intraoperative HRM along with the novel imaging tool "EndoFLIP" are safe and useful techniques in determining the adequacy of Heller's myotomy, and therefore decreasing potential future recurrence of symptoms [118–123].

Finally, dysfunction of the upper esophageal sphincter (UES), such as an increased resting pressure, shorter relaxation after swallowing, and shorter interval to pharyngeal contractions after UES relaxation, has been demonstrated in achalasia patients. The clinical significance of such findings is still not well understood [124, 125].

Management

The therapeutic management of achalasia includes pharmacological, interventional, and surgical options. None are curative but they aim in providing symptomatic relief of the EGJ obstruction without addressing the esophageal body dysmotility [126, 127]. However, there is some evidence suggesting potential improvement of the esophageal motility after surgical treatment for achalasia [128].

Pharmacological Therapy

Oral pharmacological treatment is the least effective in managing achalasia [129]. Isosorbide dinitrate and nifedipine act by relaxing the smooth muscles and by blocking the calcium channels, respectively; both reduce the LES pressure [130, 131]. They are administered prior to meals and are considered a temporary measure until a more definitive treatment, either dilatation or surgery, is provided. Oral pharmacotherapy alone is recommended in adult patients who are not willing or eligible for dilatation or surgery and in whom endoscopic botulinum toxin injection (EBTI) has previously failed [4].

Endoscopic Botulinum Toxin Injection

Intrasphincteric botulinum toxin injection blocks the presynaptic cholinergic terminals in the LES and therefore inhibits the secretion of acetylcholine at the neuromuscular junction. The latter results in chemical denervation with consequent opposition of the excitatory effect of acetylcholine and disruption of the relentless contraction of LES [4]. The experience of EBTI in the treatment of childhood achalasia is

relatively limited. The method involves the endoscopic injection of 25 units of botulinum toxin in each one of the four LES quadrants (100 units in total) [73]. Similar to pharmacotherapy, EBTI offers a short-term symptom relief, as pneumatic dilatation (PD) or Heller myotomy (HM) will be eventually required for a more sustained clinical improvement [132, 133].

Pneumatic Dilatation

The goal of performing a forceful esophageal dilatation is to stretch and disrupt the LES fibers to such an extent that it alleviates EGJ obstruction without inducing GER [4, 134]. The procedure is performed under general anesthesia, and patients undergoing dilatation need to be eligible for surgery in the unfortunate event of esophageal perforation [4]. A balloon dilator is inserted with fluoroscopic or endoscopic guidance [71, 135]. The non-radiopaque polyethylene balloon dilators are preferred over the bougienage or the standard balloon dilators as the latter are not effective enough in rupturing the LES fibers and achieving adequate symptoms' control. The modern dilators are of graded size (3.0, 3.5, 4.0 cm); however, for technical reasons PD is usually performed only in children older than 6 years of age or those weighing over 20 kg [4, 71]. After the correct position is confirmed, the balloon is distended for 60 s with pressures that vary among institutions from 2 to 12 psi [71, 135]. Balloon distention until obliteration of its waist while under fluoroscopy is more important for achieving symptom control than balloon distention time [4]. It is advisable that all patients are observed for a certain period of time prior to discharge home, and if symptomatic, they need to be assessed with a gastrografin esophagogram to exclude potential esophageal perforation. The incidence of esophageal perforation in adults who underwent PD for achalasia was reported between 4% and 12% [135]. No cases of post-PD esophageal perforations were documented in recent pediatric studies [71, 135–137]. Small asymptomatic perforations are managed conservatively (intravenous antibiotics, total parenteral nutrition), whereas immediate surgical intervention is mandated for free perforations [138, 139]. The majority of perforations occur during the first dilatation most probably due to inaccurate placement and distention of the dilator; GER has been reported in 15–35% of the patients who underwent dilatation for achalasia treatment [78, 140]. The latter is of important significance as recurrence of symptoms in post-PD patients may be due to reflux-related distal esophageal stricture. Thus, treatment with proton pump inhibitors (PPI) is warranted in post-PD patients complaining of GER symptoms. Other potential post-PD complications include aspiration pneumonia and pain that may require prolonged hospitalization [136].

PD achieves adequate control of symptoms ranging from 67% after the first dilatation to 87.5% after subsequent procedures [71, 135]. Available data suggest that although PD achieves immediate symptom relief, it bears a significant likelihood of symptoms' recurrence that will require subsequent interventions in the form of either repeat PD or surgery [73, 136, 137, 141]. It is worth mentioning that minimally invasive surgical techniques have challenged the role of PD as the first-line treatment in childhood achalasia, and the recently published data question the efficacy of PD as HM was proven to be significantly superior to dilatation [73, 136, 137, 142].

Surgery

The mainstay of surgical treatment for achalasia is Heller's myotomy, which was first described in 1913 [143]. Over the past decades, HM has evolved from the open thoracotomy to the minimally invasive laparoscopic technique, which has become the procedure of choice among surgeons due to its high efficacy, faster recovery, and minimum morbidity rate [136, 141, 142, 144–147]. Moreover, robot-assisted HM techniques have been recently described in pediatric patients and resulted in promising outcomes [148, 149]. The resolution of symptoms after HM largely depends on the length of the myotomy; this should be long enough to diminish the EGJ obstruction but not excessively long so that it induces postoperative GER. The optimal length of HM in children of different age groups has not been established yet. One report advocates an incision length between 4 and 6 cm that ends 5 mm distally to EGJ along with minimal mobilization of the adjacent anatomical structures [150]. The role of both "EndoFLIP" and intraoperative manometry appears to be very promising in determining the optimal myotomy length [118–123].

The addition of a fundoplication after HM and also the type of the anti-reflux procedure are still a matter of debate. An anti-reflux procedure reduces the risk of postoperative GER but at the same time increases the likelihood of dysphagia, as it creates a high-pressure zone to an esophageal body with an already impaired motility [151–153]. An anti-reflux procedure may be warranted when a wide mobilization of the esophagus is inevitable in order to technically facilitate the myotomy [150]. Regarding the most appropriate type of the anti-reflux procedure, there are data revealing that a loose Nissen, Toupet, Dor, or Thal fundoplication along with HM fundoplication are safe and result in good outcomes in the treatment of childhood achalasia [95, 137, 142, 145, 154, 155]. The American College of Gastroenterology advocates PPI treatment in achalasia adult patients complaining of heartburn post-HM in spite of the presence of a fundoplication [4].

Patients who have developed "end-stage" achalasia and failed myotomy are candidates for esophagectomy with gastric transposition [4, 95, 136, 137]. However, esophagectomy bears greater morbidity and mortality compared to laparoscopic HM; dysphagia requiring PD can still recur up to 50% of the adult patients that underwent esophagectomy for "end-stage" achalasia [156].

Emerging Treatments

POEM is an acronym for "peroral esophageal myotomy": it is a novel technique developed in Japan [157, 158], whereby the endoscopist performs an LES myotomy by creating a submucosal tunnel using a forward-viewing endoscope. The overall success in adult studies has been more than 80% at 12-month follow-up [158]. There are relatively limited data regarding the applicability, safety, and efficacy of this method in childhood achalasia. Recently published studies (including a systematic review with meta-analysis) revealed that POEM in pediatric patients was effective and safe, but clearly further evidence is needed in order to assess its long-term outcomes (e.g., sustained therapeutic effect, incidence of post-POEM gastroesophageal reflux disease) and its efficacy/safety compared to other therapeutic options for childhood achalasia [159–169].

There is little evidence that other techniques for treating achalasia such as removable self-expanding metallic stents are efficacious, and moreover there is a paucity of studies in children in order to evaluate the safety and efficacy of such treatments in pediatrics [170–172].

Follow-Up and Surveillance

Currently, there is a lack of consensus regarding the optimal short- and long-term follow-up of children treated for esophageal achalasia. It is, however, advisable that pediatric patients receive regular follow-up assessment to ensure a sustained relief of their pre-treatment symptomatology along with an assessment of their physical growth and overall development [172].

Patients treated for achalasia have a normal life expectancy [173]. Despite an increased risk of esophageal carcinoma in patients with achalasia [174–176], there are limited data to support regular screening for cancer. Thus, the American College of Gastroenterology, the American Society for Gastrointestinal Endoscopy, and the International Society for Diseases of the Esophagus do not advocate routine endoscopic surveillance for patients with esophageal achalasia [4, 163, 172, 177]. Nevertheless, radiological or endoscopic surveillance is recommended by numerous authorities for the assessment of adult patients treated for achalasia as they are at an increased risk for developing end-stage disease with megaesophagus. This approach with regular evaluation in 3-year intervals is particularly proposed if the disease has been diagnosed for more than 10 to 15 years [178]. Clearly, more data are required for the implementation of such recommendations in childhood achalasia.

Conclusion

Childhood esophageal achalasia is an enigmatic disease. Contemporary diagnostic modalities (upper GI contrast series, endoscopy, HRM) offer rapid and accurate diagnoses. Current data suggest that laparoscopic HM combined or not with an anti-reflux procedure seems to be the therapeutic procedure that offers the most durable and sustained long-term symptom relief.

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Helicobacter Pylori Gastritis and Peptic Ulcer Disease

12

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Abbreviations

AMO	Amoxicillin
BabA	Blood antigen-binding adhesin
CagA	Cytotoxin-associated gene A
CLA	Clarithromycin
COX-1	Cyclooxygenase-1
^{13}C-UBT	^{13}C-urea breath test
dupA	Duodenal ulcer promoting
ELISA	Enzyme-linked immunosorbent assay
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology and Nutrition
FISH	Fluorescence in situ hybridization
GGT	Gamma-glutamyl transpeptidase
H. pylori	*Helicobacter pylori*
IDA	Iron deficiency anemia
MALT	Mucosa-associated lymphoid tissue
MET	Metronidazole
NASPGHAN	North American Society for Pediatric Gastroenterology Hepatology, and Nutrition
NSAIDs	Nonsteroidal anti-inflammatory drugs
OipA	Outer inflammatory protein A
OMV	Outer membrane vesicles
PAMPs	Pathogen-associated molecular patterns
PCR	Polymerase chain reaction
PPI	Proton pump inhibitors
PRRs	Pattern recognition receptors
SabA	Sialic acid-binding adhesin
T4SS	Type IV secretion system
VacA	Vacuolating cytotoxin A
WB	Western Blot

Introduction

Helicobacter (H.) pylori is a Gram-negative, microaerophilic spiral bacterium that infects the stomach. Stomach colonization causes chronic gastritis that can remain silent, due to the dynamic equilibrium between the bacterium and its human host, or evolve into more severe diseases, such as atrophic gastritis, peptic ulcer, lymphoma of the mucosa-associated lymphoid tissue (MALT), or gastric adenocarcinoma [1, 2]. Difference in the consequences of *H. pylori* infection could be at least partially explained by the high variability of colonizing *H. pylori* strains and host response to this microbe [3].

The discovery of *H. pylori* as a major cause of peptic ulcer disease had a significant impact on its perception and treatment. The importance of the discovery was awarded by the Nobel Prize in 2005 to Marshall and Warren who proved the etiological role of *H. pylori* in gastritis and peptic ulcer disease.

Children differ from adults with respect to *H. pylori* infection in terms of the prevalence of the infection, the complication rate, the near-absence of gastric malignancies, age-specific problems with diagnostic tests and drugs, and a higher rate of antibiotic resistance [4, 5].

Bacterial Pathogenesis

H. pylori pathogenesis, still not fully elucidated, is mediated by a complex interplay between bacterial, host, and environmental factors [6, 7]. When *H. pylori* reaches the gastric lumen, in order to establish successful and persistent colonization, the bacterium employs different mechanisms (Fig. 12.1): (1). to survive under acidic stomach condition, (2). to move toward epithelium cells, (3). to attach to host cells by adhesins/receptors interaction, and (4). to cause tissue damage [6, 7].

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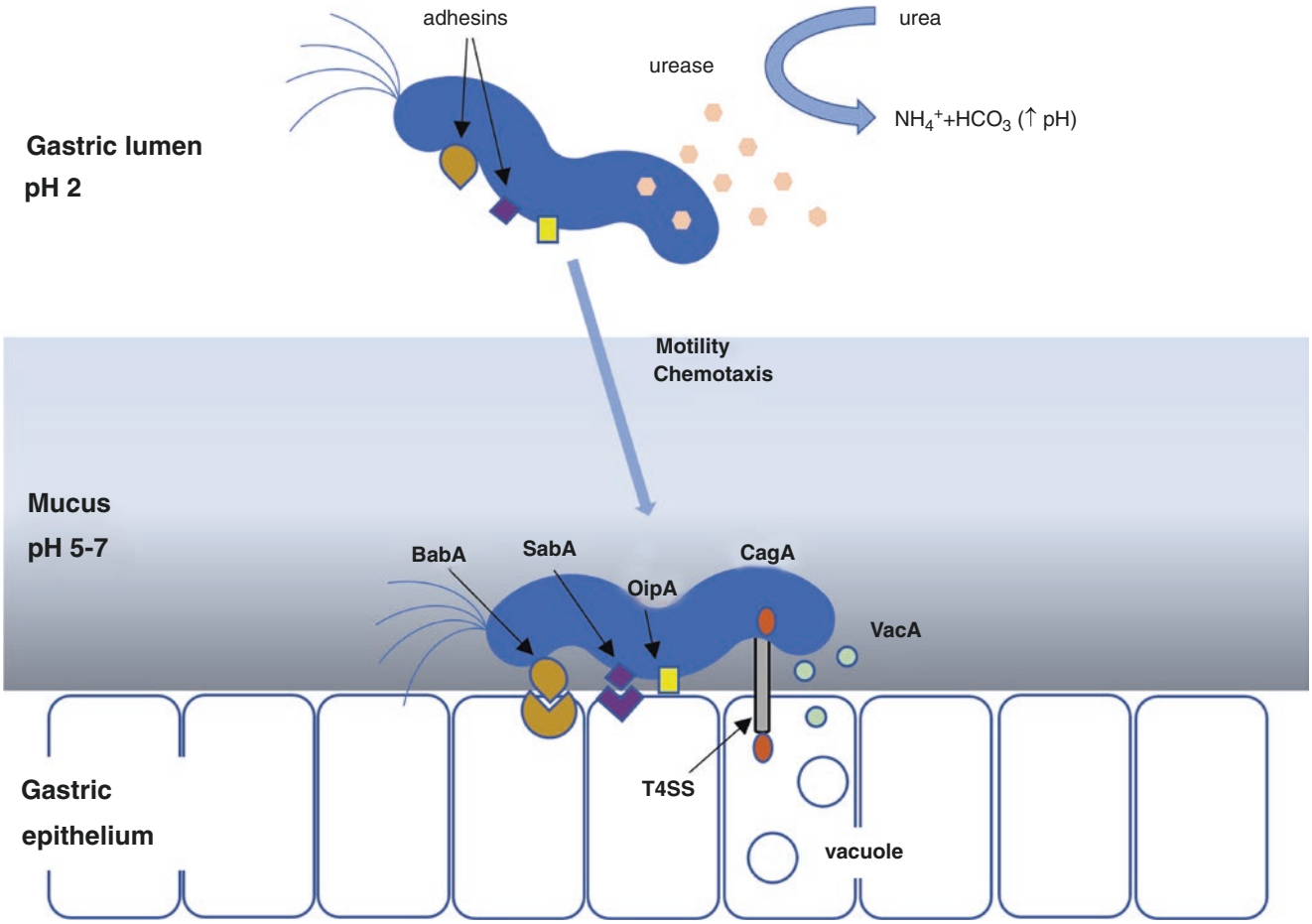

Fig. 12.1 *H. pylori*-associated factors that enable colonization and persistence. Initially, *H. pylori* produces urease which raises the pH through ammonia production. Directed by chemotaxis and enabled by bacterial helical rod-shaped and flagellar-based motility, *H. pylori* moves toward the gastric epithelium. There, colonization of the gastric mucosa is mediated by surface adhesins like SabA and BabA. Finally, *H. pylori* releases several effector proteins/toxins, causing host tissue damage. These factors include cell-bound components (OipA), externally secreted molecules (VacA), and antigens that need to be introduced to a host cell via the type IV secretion system (T4SS) (CagA protein)

Survival in the Acidic Environment

H. pylori prefers neutral or close to neutral pH (pH 5.5–7.5), and in order to survive the acidic environment of the gastric lumen where pH is around 2.0 it has developed some adjusting mechanisms. For this purpose, *H. pylori* produces large amount of urease, a nickel-containing enzyme, which requires efficient uptake of nickel from the environment for its activity. Urease converts urea to ammonia which is transported outside of bacterial cell raising the pH of the surrounding area. In addition to the urease, for ammonia production, *H. pylori* possesses also two aliphatic amidases and aspartase [7–9].

Movement Toward Epithelial Cells

Because of its characteristic helical shape and flagellar motility, *H. pylori* can swim in gastric content and can penetrate the mucus layer in corkscrew-like movement [6, 8, 9]. Four to eight sheathed flagella are situated on a single or on both poles of the bacterium [6]. The number of flagella seems to play an important role in bacterial speed during movement [9]. *H. pylori* flagella can provide different movements according to the media in which the bacterium is located: in liquid media "swimming motility," in soft agar "spreading," and on the surface of solid media, "swarming" movements are observed [6]. More than 40 proteins are involved in the biosynthesis and operation of flagella, making motility one of the most complex processes in the bacteria [7].

Besides flagella, *H. pylori* mobility also depends on chemotaxic action which allows the bacterium to control its swimming behavior and directs itself toward higher pH. *H. pylori* has four chemoreceptors, of which the transducer-like protein is the most important one, located in the bacterial membrane or in the cytoplasm [6, 9]. As a response to extracellular chemical signals (such as mucin, sodium bicarbonate, urea, sodium chloride, and some specific amino acids)

and following interaction with their respective ligands, molecular signal transduction cascade is initiated, which causes a change in the direction of rotation of the flagellar motors [6, 8, 9].

Directly adjacent to the gastric surface epithelium lies the mucus layer that has near-neutral pH [8]. *H. pylori* crosses the gastric mucus layer and starts colonizing the gastric mucosa by adhering to the gastric epithelium where necessary nutrients and protection from the host immune system are obtained [9].

Attachment to Host Cells by Adhesins/ Receptors Interaction

H. pylori colonization of the gastric mucosa is mediated by surface adhesins including blood group antigen-binding adhesin (BabA), sialic acid-binding adhesin (SabA), neutrophil-activating protein, and other adhesins. One of the most well-characterized adhesins is BabA, which binds to the Lewis b ABO blood group antigen expressed on host gastric epithelium cells [6–8]. Bacteria with high BabA expression are more virulent and carry increased risk of peptic ulcer disease and gastric cancer, although the presence of BabA is not correlated to gastric-related diseases in Asians [6, 7]. SabA adhesin plays a critical role in assisting *H. pylori* to adhere to and colonize the gastric epithelium cells of a patient with gastritis. In patients infected with SabA-positive strains, the density of *H. pylori* is dramatically higher than in patients infected with SabA-negative strains [7].

On the other hand, neutrophil-activating protein is highly associated with the chronic gastritis, and infiltration of neutrophils and mononuclear cells into the gastric mucosa [7]. Other adhesins identified are heat shock protein 60, adherence-associated proteins, *H. pylori* outer membrane protein, and lacdiNAc-binding adhesin [7].

Causing Tissue Damage

H. pylori has a number of virulence factors which influence colonization and disease severity [8, 9]. These factors include cell-bound components, externally secreted molecules, and antigens that need to be introduced to a host cell via the type IV secretion system (T4SS) (such as CagA protein) [10].

Similar to other Gram-negative bacteria, *H. pylori* produces outer membrane vesicles (OMVs) that contain various virulence factors. OMVs are continuously shed from the surface of *H. pylori* during infection. Various biologically active compounds of *H. pylori* present in OMVs can be internalized into host cells where they influence signaling pathways, promote apoptosis of gastric epithelial cells, and affect immune responses leading to disease development. The following proteins of *H. pylori* OMVs have been identified: vacuolating cytotoxin (VacA), cytotoxin-associated gene A (CagA), blood group antigen-binding adhesin, sialic acid-binding adhesin, outer inflammatory protein A (OipA), *H. pylori* neutrophil-activating protein, adherence-associated lipoprotein, and urease [10].

CagA is an oncoprotein and one of the most important and best-studied virulence factors [8, 9]. It is encoded by *cagA* gene contained in a *cag* pathogenicity island, a region that also possesses the coding sequence of a type IV secretion system (T4SS) [6, 8]. Two types of CagA protein are recognized, Western- and East Asian-type [7].

CagA can affect the host cell in several aspects, such as the formation of gastric epithelium cell pedestals, the change of the cytoskeleton, affecting the proliferation of cells, and stimulating the gastric epithelium cells to secrete IL-8, as CagA-positive strains are directly associated with acute gastritis, gastric ulcer, and gastric cancer development [7, 8].

When *H. pylori* makes contact with host cells, CagA is translocated and injected into the host cell cytoplasm (via T4SS) where it activates various oncogenic pathways [8, 9]. Within the host cell, CagA undergoes tyrosine phosphorylation at a Glu-Pro-Ile-Tyr-Ala (EPIYA) motif and consequently promotes the cell changes [6, 8, 9]. EPIYA-A and EPIYA-B segments have been found in most *cag*A-positive *H. pylori* strains, while EPIYA-C and EPIYA-D segments are related to Western and Eastern strains, respectively. Strains containing EPIYA-D are associated with a higher risk of cancer development, while among Western-type strains, the number of EPIYA-C motifs has been considered to be the most important risk factor for developing cancer [6].

However, the presence of *cagA* usually coincides with the presence of other virulence factors, which can influence one another [8].

VacA, one of the major virulence factors in *H. pylori* infection, was named for its ability to induce numerous large vacuoles in host cells [6, 8]. Unlike CagA, it forms an autotransporter structure to secrete itself without the need for host cell contact [8]. Besides vacuolation, VacA can embed into the host cell membrane, having the characteristic of an anion-selection channel that can release bicarbonate and organic anions in the host cytoplasm [7]. It can also disrupt the barrier function of epithelial cells, allowing leakage of crucial nutrients such as iron, nickel, and amino acids that likely improve *H. pylori* growth [8]. Consequently, the integrity of cell structures is destabilized, leading cells to collapse [6]. In addition, VacA disrupts the balance of cell proliferation and death by affecting genes that regulate the cell cycle; it can induce acute inflammatory responses and might also participate in inducing immune tolerance and persistent *H. pylori* infection through its activities on T-cells and antigen-presenting cells [6, 7].

All *H. pylori* strains contain *vacA* genes, but not all strains produce functional VacA. This is due to polymorphisms within the *vacA* gene, particularly at the amino terminus (s region: s1a, s1b, s1c, and s2), in the middle of the gene (m region: m1, m1T, m2), and in an intermediate region (i

region: i1, i2, i3) [7, 8]. Numerous studies have shown association of certain subtypes with different clinical pictures, although not always consistent in different parts of the world [7].

The role and the significance of *hom*A and *hom*B genes are still uncertain. It seems that *hom*A and *hom*B genes are not independent markers of *H. pylori* virulence; however, they may act synergistically with other known virulence genes, causing severe gastritis in children [11].

The prevalence of duodenal ulcer-promoting gene (*dupA)* gene was shown to be significantly higher in strains from duodenal ulcer patients than those from gastritis or gastric cancer patients [9]. Furthermore, the presence of functional *dup*A has been considered to be a protective factor for gastric carcinoma development. Besides, *dupA* seems to provide a higher acid resistance to the bacterium and might also promote an increase in the production of IL-8 that leads to mucosal inflammation and polymorphonuclear leukocyte infiltration. Interestingly, the relation between *dup*A-positive *H. pylori* strains and duodenal ulcers has been observed in Asian, but not in the Western population [6].

OipA, in synergy with other virulence factors, contributes to both adhesion and increased inflammation by inducing enhanced IL-8 production. It might be related to changes in cell proliferation, reduction of cell–cell junctions and is possibly associated with carcinogenicity [6, 8, 10].

H. pylori gamma-glutamyl transpeptidase (GGT) functions as a virulence factor, although it had not been identified as a virulence factor in other bacteria [8]. GGT is an enzyme, hydrolase, that catalyzes the conversion of glutamine into glutamate and ammonia, and the hydrolysis of glutathione into glutamate and cysteinylglycine [6]. Its activity leads to the production of reactive oxygen species, which induces cell-cycle arrest, apoptosis, and necrosis. In addition, GGT inhibits T-cell proliferation and dendritic cell differentiation, and the higher GGT activity has been observed in peptic ulcer patients [6, 8].

Immunologic Aspects

H. pylori infection induces complex host immune responses, involving both innate and adaptive mechanisms. Given the initial contact with the pathogen, various *H. pylori* antigens bind to gastric cell receptors, including toll-like receptors located on epithelial cell membranes or found in intracellular vesicles. Such interaction promotes signaling pathways followed by proinflammatory cytokine release. Besides receptor activation, injection of CagA through T4SS also leads to the production of cytokines. Subsequently, gastric mucosa is infiltrated by neutrophils and mononuclear cells. Moreover, CD4+ and CD8+ T-cells, components of adaptive immunity, are also recruited. Regarding general cytokine profiles in *H. pylori*-positive patients, studies have suggested a Th1-polarized response, characterized by scarce IL-4 and enhanced levels of gamma interferon, tumor necrosis factor, IL-1β, IL-6, IL-7, IL-8, IL-10, and IL-18. With the exception of IL-10, which seems to play a role in limiting the inflammatory response, other increased cytokines might promote proinflammatory effects during *H. pylori* infection [6].

In summary, after entering the host stomach, *H. pylori* utilizes its urease activity to neutralize the hostile acidic condition, while factors affecting movement toward host gastric epithelium cells are shape, flagella-mediated motility, chemotaxis, and adherence. Specific interactions between bacterial adhesins with host cell receptors enable successful colonization and persistent infection [7]. Finally, *H. pylori* releases several effector proteins/toxins, causing host tissue damage. In addition, host immune system is activated, further leading to the formation of clinical diseases such as gastritis and ulcer [6, 7]. Once the persistent infection is established, the bacterium stays within the stomach for lifetime unless eradicated [9].

Epidemiology and Transmission

Over the last decades, a continuous decrease in *H. pylori* prevalence has been reported from different areas around the world, and not only in developed Western countries but also in countries like China and Iran [12]. However, *H. pylori* prevalence varies between countries, with lowest prevalence of 5–10% in Western Europe and the USA and very high prevalence in developing countries of even 80% [13, 14]. An increased prevalence in developing countries is mainly due to the combined effects of poor living conditions, poor hygiene, and overcrowding [15]. In developing countries, *H. pylori* infection is acquired predominantly during early childhood, and although previously it was reported that in the more developed areas the infection gradually increases with age with the highest incidence rate in childhood and adolescence [16, 17], the recent long-term follow-up study performed in Ireland showed that *H. pylori* almost never occurs after early childhood also in developed country [18].

The modes of *H. pylori* transmission are still not entirely clarified, but person-to-person contact, especially within family members, is the most commonly implicated mechanism through oral–oral, fecal–oral, or gastric–oral route, although environmental exposure routes are also possible [19]. It has been described that transmission primarily occurs between mothers and their offspring or between siblings [17, 20]. Currently available evidence indicate that especially in populations with low *H. pylori* prevalence the infected mother is likely to be the main source for childhood *H. pylori* infection [20]. However, only few studies have longitudinally examined factors which influence *H.*

pylori transmission. In the well-designed longitudinal study, Muhsen et al. included Israeli Arab children aged 3–5 years from three villages in northern Israel which were followed up for 3–4 years [21]. Having *H. pylori*-infected sibling was identified as an independent risk factor for both early and persistent *H. pylori* infection. It was also shown that persistent *H. pylori* infection in older siblings always precedes infection in younger siblings especially if the age difference was less than 3 years [22]. In a recent DNA-based transmission study, in which stool specimens were used to genotype *H. pylori* strains, it was reported that strains were not shared between spouses while similar strains were frequently found in mother and child pairs and in siblings. However, as in some families there were members with unique strains, it was suggested that also in developed countries sources of infection outside of the immediate family may exist [23].

Due to very close interpersonal contact between children in day-care centers, it was proposed that their attendance could also be a risk factor [16]. However, a meta-analysis of 16 studies did not confirm this hypothesis, but it included studies substantially varied in the methodology; they were performed in different types of childcare, in different age groups, with different exposure durations which all resulted in a high heterogeneity in the meta-analysis [16].

There is some evidence of positive correlation between the presence of *H. pylori* in the oral cavity and gastric mucosa [24]. Moreover, it seems that eradication rate is significantly higher in stomach than in oral cavity. These results suggest that infected dental plaque, saliva, or other parts of the oral cavity may be a source for infection and reinfection of *H. pylori* through oral–oral route [24]. On the other hand, some epidemiological data do not support oral–oral transmission as cohabiting couples are infected with different strains and treated adult patients are not infected by their untreated infected partner [23].

Gastritis

Gastritis is defined as histologically confirmed inflammation of the gastric mucosa mainly composed of lymphocytes, plasma cells, histiocytes, and granulocytes within the lamina propria [25]. *H. pylori* is by far the most common etiological agent causing gastritis in adults and children. The most common site of infection is the antrum, which is an absorptive rather than a secretory region of the stomach enabling the slightly higher pH at which the organism can survive [26]. In the initial phase of *H. pylori* infection, the gastric mucosa becomes acutely inflamed with impairment in the acid secretion [27]. In large majority of patients, gastritis progresses to chronic active gastritis which is characterized by the presence of both mononuclear and neutrophilic ("active") inflammation [25]. In children, the "active" or neutrophilic count is lower than that reported in adults.

In adults, *H. pylori* infection causes three main gastritis types; mild pangastritis where inflammation evenly affects the whole stomach, antrum-predominant gastritis where the degree of inflammation is strongest in the antrum and acid secretion tends to be increased, and the most infrequent phenotype affecting only approximately 1% of infected subjects body-predominant gastritis with even atrophic changes and impaired gastric acid secretion [27]. Antrum-predominant gastritis may subsequently be complicated by duodenal ulcer, while corpus-predominant atrophic gastritis increases the risk of gastric cancer [28]. Whether this distinction between gastritis types exists already in children or it develops later in life is still unknown [29].

In addition to the topographic expression of *H. pylori*-induced chronic gastritis, the characterization of gastritis needs to include report on the activity and chronicity of inflammation and on the development of atrophic changes with or without intestinal metaplasia [30].

H. pylori gastritis has unique features in children, such as the nodular aspect of the gastric antrum, antral predominance of gastritis in most patients, and uncommon diagnosis of gastric atrophy and intestinal metaplasia. Nodular gastritis is usually characterized as an endoscopic appearance which has been described as gooseflesh-like or cobblestone markings on the gastric mucosa (Fig. 12.2) [31]. Antral nodularity seen on endoscopy is histologically associated with inflammatory cell infiltrates and lymphoid follicles, and it is the only sign with a high positive predictive value for the presence of *H. pylori* infection [32].

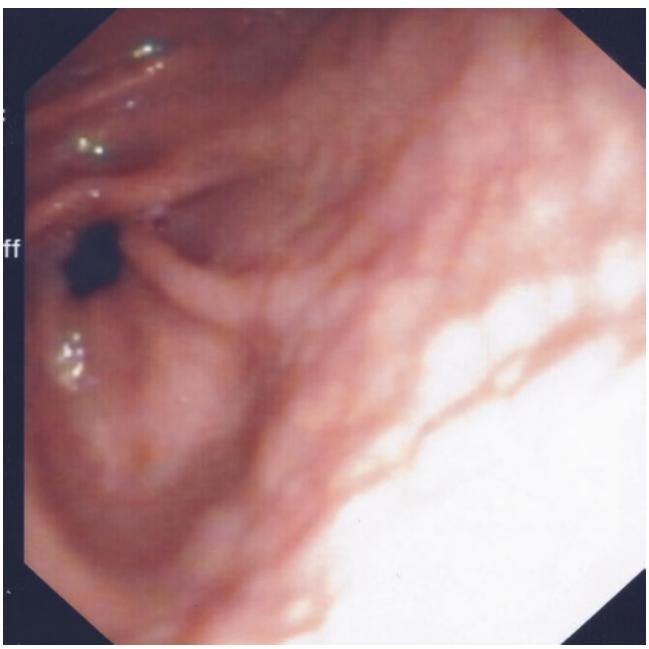

Fig. 12.2 Antral nodularity associated with *H. pylori* infection

After successful eradication therapy, the neutrophils quickly disappear, and any persistence of neutrophils and/or mononuclear infiltrate could be an indication of the treatment failure.

Peptic Ulcer Disease

Gastritis and peptic ulcer disease were previously considered as separate diseases; however, better understanding of the pathomechanisms and discovery of *H. pylori* revealed the close relation between those two entities. Peptic ulceration of the stomach or duodenum, either primary or secondary, is almost always accompanied by abnormalities of the gastric mucosa, either a gastritis or a gastropathy [33]. Peptic ulcer disease is the ultimate loss of mucosal integrity and develops when the protective mechanisms of the gastrointestinal mucosa, such as mucus and bicarbonate secretion, are overwhelmed by the damaging effects of gastric acid and pepsin [34]. By the definition, peptic ulcers are deep mucosal lesions that disrupt muscularis mucosa layer of gastric or duodenal wall. They occur in the stomach mainly in antrocorporeal mucosal transitional zone along the lesser curve or in proximal duodenum, duodenal bulb [35]. Peptic ulcers are more common in duodenum than in stomach, and *H. pylori* is the most frequent cause. Other etiological factors associated with gastritis and peptic ulcer disease are presented in Table 12.1.

Table 12.1 Causes of gastritis and peptic ulcer disease

Primary	*H. pylori*-associated *H. pylori*-negative (idiopathic) Hypersecretory states: Zollinger–Ellison syndrome G-cell hyperplasia/hyperfunction Systemic mastocytosis Cystic fibrosis Hyperparathyroidism Short bowel syndrome Renal failure
Secondary	Stress ulcers Drugs Aspirin Nonsteroidal anti-inflammatory drugs (NSAID) Corticosteroids Chemotherapy Valproic acid Alcohol Potassium chloride Immune/allergic Allergic gastritis and eosinophilic gastritis Graft-versus-host disease Henoch–Schönlein gastritis Coeliac disease Autoimmune disease Granulomatous gastritides: Crohn's disease Foreign body reaction Idiopathic Sarcoidosis Histiocytosis X Tuberculosis Menetrier disease Other infections: Helicobacter heilmannii Cytomegalovirus Phlegmonous gastritis and emphysematous Herpes simplex Influenza A Syphilis Candida albicans Histoplasmosis Mucormycosis Anisakiasis Radiation gastropathy

Available literature suggests that the lifetime risk for the development of peptic ulcer disease in *H. pylori*-positive patients ranges between 10% and 20% [36]. Peptic ulcer disease occurs less frequently in children than in adults. A large prospective European multicenter study including more than 1400 symptomatic infected children found gastric or duodenal ulcers in 3.5% of children below 6 years of age, 4.6% children aged 6–11 years, and in 10.4% of those older than 12 years [37]. Subsequent, prospective, also European multicenter study found that even 8.1% of children had ulcers or erosions [38]. In a retrospective review of 619 Chinese children who had undergone upper endoscopy for investigation of upper gastrointestinal symptoms, Tam et al. [39] have found peptic ulcers in 6.9% of children.

The discovery of *H. pylori* has turned the pathogenesis of peptic ulcer disease from a hyperacid disease to an infectious and immunopathogenetic disease where a variety of ulcer-promoting host and bacterial factors are involved in the complex pathogenesis [27]. However, despite that, gastric acid secretion remains important factor of peptic ulcer disease development. Patients with *H. pylori* gastritis and duodenal ulcers present with a much higher gastric acid output following gastrin stimulation than patients with *H. pylori* gastritis but without duodenal ulcer [40]. The mechanisms controlling acid secretion are hormonal and very complex in nature. The hormonal drive is exerted by hypergastrinemia, which was originally interpreted to result from the alkalization induced by *H. pylori.* Higher pH around G cells interrupts the negative feedback control, and they continue to release gastrin [27]. Another explanation for hypergastrinemia could be the *H. pylori*-induced impaired synthesis and release of somatostatin [27]. Somatostatin is a hormone that inhibits gastrin synthesis and subsequently inhibits acid secretion. Moreover, *H. pylori* virulence factors also contribute to development of peptic ulcer disease. In children, as in adults, *CagA* and *VacA* genes are the most frequently implicated virulence factors associated with increased risk of peptic ulcer disease [41].

In gastric ulcer, the role of acid and *H. pylori*, although certainly contributory, is of less dominance as compared to duodenal ulcer [27].

Epigastric pain or discomfort exacerbated by the meal is often a symptom of peptic ulcer disease in children; however, it may also be a presenting symptom of more common disorders such as non-ulcer dyspepsia and constipation among others [33]. Other presenting symptoms include anorexia, nausea, early satiety, recurrent vomiting, and anemia. Up to 25% of children with duodenal ulcers can present silently, approximately 25% with bleeding and antecedent pain, and the rest with abdominal pain or recurrent vomiting [33, 42]. Among all clinical signs, epigastric tenderness, pain awakening the child at night, hematemesis, melena, and stunted weight gain can be considered as significant risk factors for ulcers or erosions independently [38]. Acute gastrointestinal bleeding is the most common complication of childhood peptic ulcer disease and may occur with longstanding antecedent epigastric pain, whereas a perforated peptic ulcer occurs more rarely [39].

Other Causes of Peptic Ulcer Disease

In adult patients, the second most common cause of peptic ulcer disease is the use of nonsteroidal anti-inflammatory drugs (NSAIDs) [43]. NSAIDs inhibit cyclooxygenase-1 enzyme (COX-1) and reduce prostaglandin synthesis, consequently diminishing gastric mucosal blood flow and decreasing a production of the mucus-bicarbonate barrier [44]. In children, gastric ulcerations from NSAID ingestion are not nearly as frequent as in adults [33]. When occurs in children, NSAID-induced ulcer is typically presented as hemorrhagic antral gastropathy and ulceration of the incisura. Other drugs associated with drug-induced gastropathy and ulcers are anticoagulants and corticosteroids [45].

Other very rare causes of peptic ulcer disease in children include hypersecretory states like Zollinger–Ellison syndrome and antral G-cell hyperplasia, and should be suspected in children with severe or recurrent duodenal and gastric ulcers, resistant to proton pump inhibitors (PPI) treatment and in children with multiple ulcers. Other conditions associated with acid hypersecretion are systemic mastocytosis, short bowel syndrome during the first year after surgical resection, hyperparathyroidism, cystic fibrosis, and renal failure [46].

Stress-related ulcers usually occur 24 h after the onset of severe critical illness including shock, hypoxemia, acidosis, sepsis, burns, head injury, encephalopathy, major surgery, and multiple organ system failure [33]. Stress erosions are typically multiple and asymptomatic until cause, sometimes severe, gastrointestinal bleeding.

Gastritis and gastric ulcerations are found in 50–60% of children with Crohn's disease [47]. Focally enhanced gastritis is an inflammatory lesion often found in Crohn's disease and involves discrete inflammatory foci containing lymphocytes, histiocytes, and granulocytes [48]. Focally enhanced gastritis, although to much lower extent, could also be found in ulcerative colitis and inflammatory bowel disease unclassified.

Ménétrier's disease is a rare disorder of unknown etiology characterized by enlarged gastric rugal folds. It is a protein-losing gastropathy, clinically presented with nonspecific gastrointestinal symptoms such as nausea, vomiting, anorexia, weight loss, and hypoalbuminemia [49]. Cytomegalovirus and *H. pylori* are the most frequently found pathogens associated with this condition.

Celiac disease-associated lymphocytic gastritis is characterized by an intense lymphocytosis of the foveolar and surface epithelium and chronic inflammation in the lamina propria. Celiac disease may manifest with dyspeptic symptoms and histological changes which normalize after gluten withdrawal [50, 51].

Eosinophilic-mediated gastritis may be a presentation of food allergy (allergic gastritis) or be a primary disease (primary eosinophilic gastritis), as gastric infiltration by eosinophils is a pathological feature found in both conditions. Allergic gastritis mainly affects young infants with cow's milk protein allergy, but multiple food intolerances may also occur. Most common symptoms include vomiting, hematemesis, poor weight gain, or symptoms of delayed gastric emptying [52]. Primary eosinophilic gastritis may manifest at any age and may affect any part of the gastric wall. In mucosal form, children may present with vomiting, abdominal pain, and gastric blood loss; motility disturbances and gastric outlet obstruction may occur if muscular layer is affected [53, 54]. Serosal forms produce eosinophilic ascites and peritonitis [55]. Eosinophilic gastritis may also be a part of the eosinophilic gastroenteritis.

Other causes of primary and secondary gastritis and peptic ulcer disease in children are presented in Table 12.1.

Malignancy

H. pylori is considered the leading cause of gastric cancer worldwide and because of that, in adult population, it is listed as a number one carcinogen. A causal relation between *H. pylori* infection and the risk of gastric malignancies, including cancer and MALT lymphoma, has been clearly proved. However, in children *H. pylori*-related malignancy is extremely rare even in Japan where gastric cancer is prevalent in adults [56]. Evidence increasingly indicates that *H. pylori*-related gastric carcinogenesis is likely to be the result of a well-choreographed interaction between the pathogen

and host, which is dependent on strain-specific bacterial factors, host genotypic traits, and permissive environmental factors [2]. Various factors influence malignant potential including age of infection, bacterial genotype, host immune response, and host genetics. It has been suggested that chronic gastritis, gastric atrophy, intestinal metaplasia, and gastric cancer develop progressively, stepwise over decades, in predisposed individuals infected with *H. pylori* [57].

Incomplete intestinal metaplasia has been described in the gastric mucosa of *H. pylori*-infected children, suggesting that it can develop even during childhood and evolve into complete intestinal metaplasia in adults [58]. Moreover, other studies have reported a significant incidence of gastric atrophy (42–55%) and intestinal metaplasia (13–21%) in children [59]. Interestingly, a higher incidence of atrophic gastritis has been observed in children from countries with high incidence of gastric cancer [60, 61]. Moreover, current evidence suggests that in high-risk populations the eradication of *H. pylori* may have the potential to decrease the risk of gastric cancer [62]. There are certain *H. pylori* genotypes associated with more severe inflammation of gastric mucosa in pediatric patients including CagA, VacA, and BabA, and their detection could be of importance in areas with high risk of carcinoma [63]. In support of this is a report which found that in high-risk population even children have a high prevalence of *H. pylori* virulence markers—CagA and VacA [64].

In the pediatric population, there are only a few studies with a small number of patients regarding the association between *H. pylori* infection and precancerous lesions in both gastric antrum and corpus [59]. There are reports which presented children with *H. pylori* infection who subsequently developed high-grade B-cell lymphoma which resolved after *H. pylori* eradication even without chemotherapy [65, 66]. All these data indicate that *H. pylori* could be associated with malignancies in children; however, the risk seems to be substantially lower than in adults [67].

Clinical Presentation of *H. pylori* Infection

Recurrent Abdominal Pain Abdominal complaints including pain, cramps, and nausea are very common in pediatric population. Usually, they are unspecific and can be symptoms of various organic diseases but are very often caused by functional gastrointestinal disorders. If a child with recurrent abdominal pain has no alarm signs or symptoms, it is most likely that the pain is functional pain. Alarm signs include persistent right upper or right lower quadrant pain, dysphagia, odynophagia, persistent vomiting, gastrointestinal blood loss, involuntary weight loss, deceleration of linear growth, delayed puberty, unexplained fever, and a family history of inflammatory bowel disease, celiac disease, or peptic ulcer disease. Whether *H. pylori* infection without peptic ulcer disease can cause recurrent abdominal pain remains unclear. Large epidemiological studies found association between recurrent abdominal pain and different social and familial factors like single-parent household, family history of peptic ulcer disease, or functional pain but not to *H. pylori* status of the child [68]. A performed meta-analysis on the relationship between recurrent abdominal pain and *H. pylori* infection in children included 38 case–control, cross-sectional and prospective studies and found no evidence for relation between recurrent abdominal pain and *H. pylori* infection in children [69]. The only pain type found associated with *H. pylori* infection was epigastric pain [69]. Several interventional but uncontrolled studies showed improvement of symptoms after the eradication of *H. pylori*; however, these studies have several biases; treatment success was not monitored and eradication of the bacteria not reported, and in other studies follow-up period was very short, for a few weeks only [70–73]. Based on these results, there is not enough evidence to support a causal relation between *H. pylori* gastritis and abdominal pain in the absence of peptic ulcer disease. Searching for *H. pylori* is, therefore, not recommended in children that otherwise fulfill the criteria of functional abdominal pain, unless upper endoscopy is performed during the diagnostic work-up in search for organic disease [5, 63].

Iron Deficiency Anemia Iron deficiency anemia (IDA) is common in children and adolescents and has variety of causes. Although previously suspected, as demonstrated in several well-conducted studies, there is no causal relationship between IDA and *H. pylori*. In children with refractory iron deficiency anemia who have not responded to initial iron supplementation therapy or had a rapid relapse, there is a possibility that anemia may be due to blood loss associated with peptic ulcer disease or iron utilization by the bacteria. Hence, if there is a clinical indication for upper endoscopy (if the child has alarm symptoms or other causes of iron deficiency anemia such as celiac disease or chronic inflammatory diseases are to be excluded), it is suggested that biopsies to confirm or rule out the presence of *H. pylori* infection may be considered. Noninvasive testing for *H. pylori* in the case of refractory IDA is not recommended. This is in agreement with clinical guidelines by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and by the North American Society for Pediatric Gastroenterology Hepatology and Nutrition (NASPGHAN) [5].

Growth There are a number of longitudinal studies that support the hypothesis that *H. pylori* infection might influence growth rate in children [74]; however, none are adequately

Table 12.2 Clinical indication for testing for *H. pylori* infection (based on ESPGHAN/NASPGHAN recommendations [5])

Justified testing for *H. pylori*	Gastrointestinal symptoms suggestive of organic disease, serious enough to justify upper endoscopy Gastric and duodenal ulcers
Considered testing for *H. pylori*	Children with refractory iron deficiency anemia when other causes have been ruled out Idiopathic thrombocytopenic purpura
Not justified testing for *H. pylori*	Functional abdominal pain Upper respiratory tract infections Periodontal disease Food allergy Sudden infant death syndrome Growth impairment

designed to evaluate confounders of growth including other gastrointestinal infections in childhood, socioeconomic status, living conditions, etc. Despite new information concerning the effect of *H. pylori* infection on poor growth, the need for well-designed studies remains. Based on published data, there is insufficient evidence that *H. pylori* infection is causally related to growth impairment in childhood [4]. Therefore, it is not recommended to test for *H. pylori* infection when investigating causes of short stature [5].

Chronic idiopathic thrombocytopenic purpura. The role of *H. pylori* in the development of chronic idiopathic thrombocytopenic purpura continues to evolve. *H. pylori* infection has been proposed to be associated with thrombocytopenic purpura based on a significantly increased platelets count following *H. pylori* eradication in approximately half of adult patients [75]. Data on children are limited; however, recent studies have implicated that eradication of *H. pylori* could induce a better treatment response in chronic idiopathic thrombocytopenic purpura [76]. Therefore, it is suggested that noninvasive diagnostic testing for *H. pylori* infection should be employed. If the noninvasive test is positive, it has to be decided individually, based on the platelet count, whether an upper endoscopy is needed before prescribing eradication therapy [5] (Table 12.2).

Diagnostic Procedures

According to ESPGHAN/NASPGHAN evidence-based guidelines for *H. pylori* infection in children, diagnostic procedures should aim to determine underlying disease and not to detect *H. pylori* [5]. Current recommendations for children do not approve "test-and-treat" approach recommended for adults [5].

Tests that detect *H. pylori* are divided into noninvasive and invasive tests. The invasive tests are used in clinical practice and the noninvasive ones mostly in epidemiology and to assess the outcome of an eradication treatment. Invasive tests require endoscopy and gastric tissue biopsy for detecting the bacterium and include culture, rapid urease test, histopathology, polymerase chain reaction (PCR), and fluorescence in situ hybridization (FISH) tests [77]. On the other hand, noninvasive tests include different methods for the detection of *H. pylori* antigens in stool, detection of antibodies against *H. pylori* in different biological materials including serum, urine, and oral samples, and widely used ^{13}C-urea breath test (^{13}C-UBT) [77].

The initial diagnosis of *H. pylori* infection should be performed using invasive gastric biopsy-based methods and should be based on positive bacterial culture or *H. pylori* gastritis on histopathology (using the updated Sydney classification) plus at least one other positive test such as rapid urease test or molecular-based assays (PCR or FISH). The initial diagnosis of *H. pylori* infection should not be based on noninvasive tests (i.e., ^{13}C-UBT, *H. pylori* stool antigen test) or other noninvasive methods. A positive noninvasive test, however, supports the diagnosis in cases in which positive histology is the only invasive test available.

Noninvasive Tests

Among noninvasive diagnostic tests, stool antigen test and ^{13}C-UBT have higher accuracy than serological or urinary antibody-based tests [78], and current guidelines recommend against using antibody-based tests for *H. pylori* in serum, whole blood, urine, and saliva in the clinical setting [5].

^{13}C-urea Breath Test For this test, patient swallows carbon-labeled urea, the non-radioactive ^{13}C (the use of low radioactive 14C-UBT is not recommended in children). If the patient is infected, the labeled urea is metabolized by the urease produced by *H. pylori* and, using the mass spectrometer, the increase of the expired $^{13}CO_2$ is detected [5]. Meta-analysis on the performance of the ^{13}C-UBT showed good accuracy in children older than 6 years of age (sensitivity 96.6%, specificity 97.7%) [79]. A crucial question for all tests performed in a pediatric population is whether the accuracy of the applied method is influenced by the age of the tested child. ^{13}C-UBT requires patient cooperation; moreover, patients need to fast before testing and drink without tracer withhold in the mouth. All that makes ^{13}C-UBT difficult to perform in children younger than 6 years of age and may at least in part explain the lower specificity reported in young children [80, 81].

Stool Antigen Tests Because *H. pylori* and its macromolecules such as proteins and DNA are shed in feces, stool-based tests have become developed [82]. Several commercial enzyme-linked immunosorbent assay (ELISA) tests for *H. pylori* stool antigens are available. The main differences

among these tests are the nature of the detecting antibodies; some kits use a polyclonal anti–*H. pylori* antibody, whereas other assays use monoclonal antibodies [82]. Meta-analysis on stool antigen-detection tests revealed that ELISA monoclonal antibodies have the best performance, with sensitivity and specificity of 97% compared to ELISA polyclonal antibodies (sensitivity of 92%, specificity of 93%), and to one-step monoclonal antibody tests (sensitivity of 88%, specificity of 93%) [82]. So far, two-step monoclonal stool antigen test has achieved the accuracy of the ^{13}C-UBT, which is considered the reference standard of the noninvasive tests [5]. Use of stool tests is generally more convenient in pediatric patients than the ^{13}C-UBT. It has several advantages: stool samples can be obtained from children without their active collaboration and are transportable by mail for analysis, the cost is lower compared to ^{13}C-UBT, and it is not age-dependent; therefore, validation studies in adults may be extrapolated to children [4].

Detection of the Antibodies The systemic immune response against *H. pylori* typically shows a transient rise in specific IgM antibodies, followed by a rise in IgG and IgA antibodies that persist during infection [83]. Since IgM antibodies against *H. pylori* are detected only transiently, they have little value for the serological diagnosis of infection [84]. Many tests based on the detection of antibodies in serum, saliva, and urine are commercially available, easy to perform, and inexpensive. However, the main problems are low sensitivity and test-to-test variability [5]. Current serologic tests are not useful for monitoring eradication of infection after therapy since they cannot distinguish between current or past *H. pylori* infection, and the antibody titers usually remain positive several months after the infection has been eradicated [83]. Taking that into account, tests based on the detection of antibodies (IgG, IgA) against *H. pylori* in serum, whole blood, urine, and saliva are not reliable for use in the clinical setting [5].

Invasive Tests

Although noninvasive tests yield high sensitivity and specificity, endoscopy with biopsies remains the only method that can detect lesions associated with the infection but also other possible causes of the patient's symptoms [77]. In *H. pylori* infection, endoscopy may show normal gastric mucosa or reveal erythema, erosions, ulcers, and, especially in children, antral nodularity.

The site from which a biopsy is taken and the number of biopsies affect the accuracy of *H. pylori* diagnosis. Normally, the highest bacterial count is found in the antrum; because of that the optimal biopsy site is the mid-antrum at the lesser curvature [85]; however, in cases of low gastric acidity and during the PPI treatment, the bacteria may be present only in the gastric body.

Furthermore, due to patchy density of *H. pylori*, the sensitivity increases with the number of biopsies taken.

At least six gastric biopsies should be taken for the initial diagnosis of *H. pylori* infection: two biopsies from the antrum and two from the corpus for the histopathological evaluation (applying the updated Sydney classification), at least one biopsy from the antrum and one from the corpus for culture (if available) and at least one biopsy for any additional diagnostic tests from the antrum (rapid urease or molecular-based assays) [5].

Regarding histopathology, the updated Sydney system for the gastritis classification emphasizes the importance of combining topographical, morphological, and etiological information in order to generate reproducible and clinically useful diagnoses. Classification is based on the location of gastritis and the presence of different histological parameters including inflammation, activity, atrophy, intestinal metaplasia, and *H. pylori* infection which are graded as mild, moderate, and marked [30]. Updated Sydney classification was not assessed in pediatric patients; however, it can help in order to unify the histopathology reports.

Biopsies should be stained with hematoxylin and eosin because this is the best method to detect atrophy and intestinal metaplasia. Special staining (Giemsa or silver stain) and immunohistochemistry should be used for the detection of *H. pylori* (Fig. 12.3). In children, hematoxylin and eosin and Giemsa stains have a sensitivity of 82% and specificity of 95% in detecting *H. pylori* gastritis [86]. With decreasing prevalence of the infection in pediatric populations in many areas of Europe and North America, the positive predictive values of the diagnostic test results decrease; test with a sen-

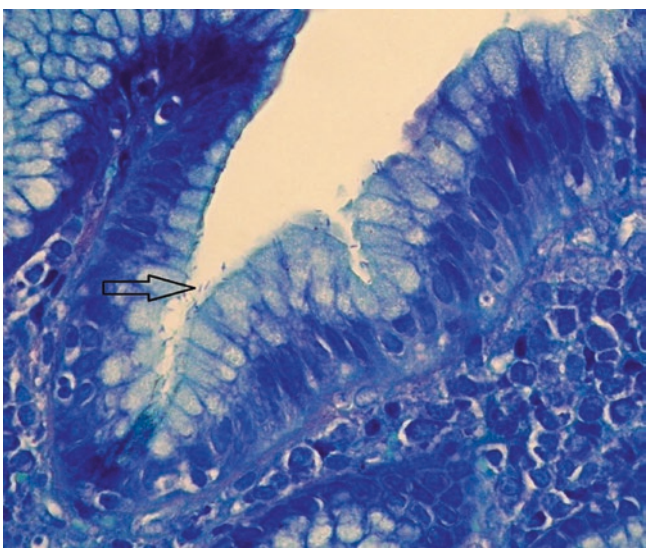

Fig. 12.3 Giemsa staining demonstrates gastric *H. pylori* bacteria (arrow)

sitivity of 90% has a positive predictive value of only 50% if the prevalence of the infection in the population is 10% [4]. Sensitivity for histology ranges from 66% to 100% and for rapid urease tests performed on the biopsies from 75% to 100% [77]. The only method that consistently has 100% specificity is culture, but sensitivity varies depending on the experience of the laboratory [77]. Current guidelines recommend that antimicrobial susceptibility of *H. pylori* should be obtained to tailor the eradication treatment accordingly. It is important to note that before testing for *H. pylori*, it should be waited for at least 2 weeks after stopping PPIs and 4 weeks after stopping antibiotics. If the culture is negative or not available, it is recommended that the diagnosis of *H. pylori* infection should be based on two tests and not only on a positive histology [5].

In summary, current guidelines recommend endoscopy with gastric biopsies from the gastric antrum and body and confirmation of infection with either positive bacterial culture or positive histopathology plus one other positive test [5].

Treatment for *Helicobacter pylori* Infection

Updated evidence-based guidelines from ESPGHAN and NASPGHAN recommend treatment for *H. pylori* in all children in whom peptic ulcer disease was detected during endoscopy [5]. Whether it is necessary to eradicate *H. pylori* in children with gastritis but without peptic ulcer disease remains questionable because there is not enough evidence which proved that bacterium eradication improves abdominal complaints in the absence of peptic ulcer. Therefore, the decision to treat *H. pylori*-associated gastritis without duodenal or gastric ulcer is subject to the judgment of the clinician and the patient and family, taking into consideration the potential risks and benefits of the treatment in every individual patient.

First-Line Treatment

First-line eradication therapy has four different regimens: (1). triple therapy with a PPI and amoxicillin and metronidazole or clarithromycin; (2). quadruple bismuth salts with PPI, amoxicillin, and metronidazole; (3). sequential therapy; and (4). concomitant therapy (Tables 12.3 and 12.4) [5].

Standard triple therapy, a combination of two antibiotics and a PPI, has been recommended as a first-line therapy since the first published pediatric guidelines. The goal of treatment is at least a 90% eradication rate at the first attempt [5]. However, reported eradication rate is much lower mainly due to antibiotic resistance. Antibiotic resistance is mostly found in clarithromycin and metronidazole. The overall incidence of clarithromycin resistance in children in Western countries is high, and current reports indicate prevalence of more than 20% in treatment-naïve patients [37, 87–90] and resistance rate is even much higher in some countries like China where clarithromycin resistance rate was higher than 80% [91]. Similarly, some areas have also high levels of resistance rate to metronidazole, more than 20% [37, 87, 91,

Table 12.3 First-line treatment options for *H. pylori* infection in children (based on ESPGHAN/NASPGHAN recommendations [5])

H. pylori susceptibility	Suggested treatment		
	Medication	Dosage	Duration
Known susceptibility			
Susceptible to CLA + susceptible to MET	PPI + AMO + CLA[a] or sequential therapy	Standard dose	14 days 10 days
Resistant to CLA + susceptible to MET	PPI + AMO + MET[a] or Bismuth-based[b]	Standard dose	14 days
Resistant to MET + susceptible to CLA	PPI + AMO + CLA[a] or Bismuth-based	Standard dose	14 days
Resistant to MET + resistant to CLA	PPI + AMO + MET[a] or Bismuth-based[b]	High dose for AMO	14 days
Unknown susceptibility	PPI + AMO + MET[a] or Bismuth-based[b] or Concomitant therapy (PPI + AMO + MET+CLA)	High dose for AMO	14 days

PPI proton pump inhibitor, *AMO* amoxicillin, *CLA* clarithromycin, *MET* metronidazole

[a]In case of penicillin allergy, if the strain is susceptible to CLA and MET, standard triple therapy with MET in place of AMO can be used; if the strain is resistant to CLA, use bismuth-based therapy with tetracycline instead of AMO in older than 8 years

[b]Bismuth-based—in children <10 years 262 mg QID, in children >10 years old 524 mg QID

Table 12.4 Dosage regimen for *H. pylori* eradication in pediatric patients (based on ESPGHAN/NASPGHAN recommendations [5])

	PPI		AMO		MET		CLA	
	Morning dose	Evening dose	Morning dose	Evening dose	Morning dose	Evening dose	Morning dose	Evening dose
Standard dose								
15–24 kg	20 mg	20 mg	500 mg	500 mg	250 mg	250 mg	250 mg	250 mg
25–34 kg	30 mg	30 mg	750 mg	750 mg	500 mg	250 mg	500 mg	250 mg
>35 kg	40 mg	40 mg	1000 mg	1000 mg	500 mg	500 mg	500 mg	500 mg
High dose								
15–24 kg			750 mg	750 mg				
25–34 kg			1000 mg	1000 mg				
>35 kg			1500 mg	1500 mg				

PPI proton pump inhibitor, *AMO* amoxicillin, *CLA* clarithromycin, *MET* metronidazole

92]. In a meta-analysis of 23 studies (with specified pediatric age groups) published in 2018, the prevalence of resistance to clarithromycin in children was from 10% in Eastern Mediterranean region and 19% in Americas region to 85% in Western Pacific region; to metronidazole from 20% in Europe to 40% in Americas region and 81% in Eastern Mediterranean region, and to levofloxacin from 4% in Europe region to 29% in Eastern Mediterranean region [93]. Based on the negative effect of antibiotic resistance on treatment outcomes, the rates of resistance in the area where the child lives should be taken into account when deciding on the initial therapeutic regimen for eradication [5]. In areas with high or unknown primary antibiotic resistance rate, culture and susceptibility testing should be performed in order to select proper treatment regimen [5].

Decreasing eradication rates with these standard triple regimens have led to the development of alternative treatment options, like sequential therapy, bismuth-based therapy, and concomitant therapy.

Sequential therapy is a two-step, 10-day therapy typically consisting of a PPI combined with amoxicillin given for the first 5 days, followed by a triple therapy including a PPI, clarithromycin, and metronidazole/tinidazole for another 5 days [94]. Meta-analysis comparing sequential to standard triple therapy found that sequential therapy is superior to 7-day standard triple therapy, however, not significantly better than 10-day or 14-day triple therapy [94]. However, more recent studies performed in Europe showed that eradication rates with sequential therapy were only 56% when clarithromycin resistance was present, while in case of strains susceptible to both clarithromycin and metronidazole primary eradication rates with a high-dose sequential 10-day regimen in children were 85.8% in the intention to treat analysis [5]. Therefore, current guidelines suggest that sequential therapy should not be given if the strain is resistant to metronidazole or clarithromycin, or if susceptibility testing is not available [5].

Bismuth-based quadruple therapy is also recommended as an alternative first-line therapy. Well-designed, randomized, multicenter studies of *H. pylori* eradication in children comparing bismuth-based regimens to the alternative recommended first-line therapies are lacking, but studies performed in adults indicate that bismuth-based therapies are effective. The results from the pediatric European register for treatment of *H. pylori* showed that when given as first treatment, bismuth-containing triple therapies were more efficacious than those containing PPI (77% versus 64%) [95, 96]. More recent studies evaluated bismuth-based quadruple therapies and showed higher eradication rates than standard triple therapy. According to current guidelines, it is recommended that bismuth quadruple therapy can be used in children if *H. pylori* antimicrobial susceptibility is unknown or in the setting of dual resistance to clarithromycin and metronidazole [5].

Concerning concomitant therapy, it was shown for adult patients that concomitant quadruple therapy (PPI and amoxicillin, metronidazole, and clarithromycin given for 10 to 14 days) was one of the most effective treatments, with a high eradication rate and acceptable frequency of adverse events. Currently, no studies assessing concomitant therapy in the pediatric setting are available. However, in children with primary double resistance to clarithromycin and metronidazole, concomitant therapy may be an option [5].

The duration of eradication therapy is still controversial. There are limited well-designed studies specifically addressing the optimal treatment duration in children. However, in adults, a recent systematic review showed that 14-day duration of treatment improves eradication rates compared to 10-day, and both are superior to 7-day treatment. Therefore, it is recommended that the duration of triple therapy should be 14 days [5]. It has been reported that, especially taking into account compliance rate, there is no benefit from longer duration of therapy [96, 97].

Rescue Therapy in Children Who Failed First-Line Treatment (Table 12.5).

Emerging evidence suggests the development of secondary antibiotic resistance in children who failed initial eradication therapy [98]. Therefore, when *H. pylori* treatment fails, rescue therapy should be individualized considering antibiotic susceptibility, the age of the child, and available

Table 12.5 Rescue therapies (after the failure of first-line therapy) for treating *H. pylori* infection in pediatric patients (based on ESPGHAN/NASPGHAN recommendations [5])

Initial antibiotic susceptibility	Past treatment regimen	Rescue treatment
Susceptible to CLA + susceptible to MET	Triple therapy using AMO + CLA Triple therapy using AMO + MET Sequential therapy	Triple therapy using AMO + MET Triple therapy using AMO + CLA Tailored treatment for 14 days after second endoscopy or treat like double resistance (Table 12.3)[a]
Resistant to CLA	Triple therapy using AMO + MET	Treat like double resistance (Table 12.3)[a]
Resistant to MET	Triple therapy using AMO + CLA	Tailored treatment for 14 days after second endoscopy or treat like double resistance[a] (Table 12.3)[a]
Unknown primary antimicrobial susceptibility	Triple therapy or sequential therapy	Tailored treatment for 14 days after second endoscopy or treat like double resistance (Table 12.3)[a]

PPI proton pump inhibitor, *AMO* amoxicillin, *CLA* clarithromycin, *MET* metronidazole
[a]In adolescents, levofloxacin or tetracycline may be considered

antimicrobial options. If possible, primary culture with antibiotic sensitivity testing should be performed to guide second-line therapy. If culture (and standard susceptibility testing) is not possible, molecular tests (including FISH) can be used to detect *H. pylori* and clarithromycin and/or fluoroquinolone resistance in gastric biopsies [78]. If primary culture and sensitivity testing are not available, when deciding on second-line therapy the initial therapy should be taken into account with avoidance of previously given regimens [5, 99]. As studies suggest that increasing acid suppression and antibiotic dose (metronidazole and amoxicillin) may improve efficacy of eradication therapy, using such regimens may be considered in children. Also, based on efficacy proved in adults, regimens using levofloxacin or tetracycline may be applied in adolescents.

Another option as a salvage therapy may be a quadruple therapy which consists of PPI, metronidazole, amoxicillin, and bismuth [5]. However, this regimen is complicated to administer, and bismuth salts are not widely available.

Although the studies on the ideal duration of therapy for second-line treatment are not conclusive, a longer duration of therapy of up to 14 days is recommended [5].

Assessment of Eradication

All children who received treatment for *H. pylori*, even if they have no symptoms, should undergo the evaluation of the treatment success. The absence of symptoms is not reliable and does not necessarily mean the *H. pylori* is eradicated. The recommended tests for *H. pylori* eradication assessment are noninvasive tests: ^{13}C-UBT and a two-step monoclonal ELISA test for the detection of *H. pylori* antigen in stool [5]. As previously presented these tests have a high sensitivity and high specificity and are not invasive. A follow-up endoscopy is not routinely indicated unless other causes of ulceration are suspected or if biopsies are needed for culture and antibiotic susceptibility testing [5].

Eradication therapy reduces the amount of *H. pylori* in the stomach, even when eradication failed because that antibiotic or PPI therapy can cause false-negative test results [100]. Therefore, assessment of eradication should be performed with noninvasive test at least 4 weeks following completion of therapy [5].

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Ménétrier Disease in Children

13

Jasmina Kikilion, Elvira Ingrid Levy, and Yvan Vandenplas

Abbreviations

HP	*Helicobacter pylori*
MD	Ménétrier disease
TGF-α	Transforming growth factor alpha

Introduction

Ménétrier disease (MD) was described by the French pathologist Pierre Ménétrier in 1888 [1, 2]. MD is an uncommon acquired self-limiting disorder in children [3, 4]. Pathogenesis and etiology are not yet fully understood [4].

Up to now, there are only approximately 60 cases of children with MD reported in literature [3, 4]. Most of these are case series. In this chapter, we discuss etiology and propose guidance to diagnosis and management.

Clinical Manifestations

Since there are no pathognomonic features described to diagnose MD, it continues to be a clinicopathological diagnosis. Symptoms described in adults (males more often affected than females) include vomiting, nausea, abdominal pain, diarrhea, weight loss, malnutrition, and peripheral edema secondary to hypo-albuminemia [1, 5]. In children, there is often a prodromal faze caused by a transient viral infection, followed by edema and gastrointestinal symptoms including emesis, epigastric pain, anorexia, diarrhea, vomiting, and abdominal pain (Table 13.1) [3, 4]. Edema is caused by hypo-albuminemia as a result of protein-losing edema of the gastric mucosa [4]. The average age of affected children is 2–5 years [6], but a case series from Gökçe et al. describes two cases of neonatal MD, both presenting with edema as major symptom [4]. As in children a spontaneous remission is common, it is possible that the disease is associated with *Helicobacter pylori* (HP) infection or transient infections such as cytomegalovirus (CMV) [4, 5]. These associations will be discussed later in this review.

There is a wide variation in clinical manifestations depending on the age of the patient. It is important to list MD in the differential diagnoses of edema occurring in combination with gastrointestinal symptoms.

Table 13.1 Menetrier disease in summary in children [1–9]

Triggers	Herpes simplex virus, Giardia lamblia, Mycoplasma pneumonia, CMV[a], and HP[b]
Symptoms	Edema, emesis, epigastric pain, anorexia, diarrhea, vomiting, and abdominal pain
Diagnostics	Endoscopy in combination of biopsy and cultures
Treatment	Self-limiting Supportive therapies: Albumin, diuretics, fluid restriction, high-protein diet, acid inhibitors, ganciclovir

[a]*CMV* Cytomegalovirus
[b]*HP Helicobacter pylori*

Pathophysiology and Etiology

The pathogenesis of MD is not yet fully understood [3, 4]. Observational studies in transgenic mice showed a relation between the possible overexpression of transforming growth factor alpha (TGF-α) and the development of gastric changes that are characteristic of MD [5]. TGF-α inhibits gastric acid production and stimulates growth of gastric epithelial cells [1]. TGF-α is a ligand that mediates signal transduction by binding epidermal growth factor receptor (EGFR), which leads to increased cellular proliferation [5]. More specifically in MD disease, overexpression of TGF-α redirects the gastric progenitor cells to surface mucous cell differentiation

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at the disadvantage of parietal and chief cell differentiation [5]. Remarkable is the observation that the gastrin levels in serum are normal, despite lower gastric acidity, which is a stimulus for increased production of gastrin [1].

In children, MD disease is transient and in general it is believed to be associated with infections such as herpes simplex virus, Giardia lamblia, Mycoplasma pneumonia, CMV, and HP [3–6]. Possible pathogenic mechanism is damage of the gastric mucosa caused by infection, which may lead to the production of abnormal local TGF-α [3]. CMV infection in the stomach causes elevation of intracellular messengers and activation of proto-oncogenes that causes an increase in the production of TGF-α in mucosal cells [6]. Some case reports show an association with some medications and allergies [4]. Several cases show MD with CMV and HP co-infection, although they propose that HP has the most causative role in the disease [4, 7]. However, given the high incidence of HP infection, these associations may just be by coincidence. A case series of two siblings with CMV-associated MD proposes the hypothesis that genetic factors could stimulate an increased production of TNF-⟨ in response to CMV infection [6].

A unique, fourth-generation pedigree with autosomal dominant gastropathy exhibiting the typical clinical, endoscopic, and pathological MD-like findings, though in the absence of protein loss and with no increase in the levels of gastric TGF-α, proposes a genetic predisposition to develop MD [8].

In conclusion, the pathogenesis has still to be explored further; however, there is evidence of overexpression of TGF-α with transformation of the gastric mucosa which is possibly mediated by genetics and provoked by an infectious trigger.

Diagnosis and Histological Findings

Diagnosis of MD starts with a thorough history of the patient, in which contact with family members with possible HP infection must be investigated. To confirm the diagnosis of MD, gastroscopy, biopsies, and cultures must be performed. Endoscopic findings are thickened gastric mucosal folds, and these are predominantly present in the body and the fundus of the stomach, relative sparing the antrum (Table 13.1) [5]. The most striking feature of MD, a histological sine qua non, is foveolar hyperplasia (expansion of the surface mucous cells) that leads to thickening of the gastric mucosa. There is a loss of parietal cells due to atrophic oxyntic glands, which secondarily leads to an increase of the gastric pH (normal pH of gastric fluid is 1–3, but in MD pH is rather 4–7) [1, 5]. Additionally, deep glands are often dilated, forming cysts. Histologically, there is a chronic inflammatory cell infiltration at the lamina propria with the presence of eosinophils and plasma cells, hyperplasia of smooth muscle, and edema [1, 5].

Other diseases with similar endoscopic findings are hypertrophic lymphocytic gastritis, eosinophilic gastritis, Zollinger–Ellison syndrome, polyposis syndrome, gastric malignancies, and lymphoma [5, 6]. To investigate a possible association of juvenile polyposis syndrome with MD, a new mechanism that involves TGF-α-SMAD 4 pathway inactivation and TGF-α overexpression related to HP infection has been proposed [8].

Concluding, the golden standard for the diagnosis of MD is to perform gastroscopy with biopsy and the typical histological findings.

Treatment

Management of MD in children is often supportive as most of the cases that are reported are associated with transient infections. As infection resolves spontaneously, MD usually resolves within several weeks to months [4, 5]. If there is evidence of HP infection, eradication can be considered, although there is a case described where MD resolved without the use of antibiotics [3, 6]. As HP is the only causative organism described that is not a transient infection, we think of the possibility that the association of MD and HP is a coincidence.

Supportive treatment includes albumin infusion to correct the hypo-albuminemia and diuretics, fluid restriction, and high-protein diet [2, 3]. Acid inhibitors such as proton pump inhibitors and H2 receptor blockers and anticholinergic agents are used to protect the stomach. Preference for acid inhibitors was not reported. Ganciclovir treatment can be considered if there is evidence for active CMV infection and if the patient is immunocompromised, very young or if spontaneous improvement does not occur [4, 6].

In adults and adolescents with chronic and severe diseases, chirurgical therapy like partial or total gastrectomy can be considered [2, 5]. Further clinical trials with cetuximab, an immunoglobulin that binds to epidermal growth factor receptor and prevents binding of TGF-α, showed promising results with rapid improvement of symptoms after the first administration in adults [1].

In conclusion, the treatment of MD in children is mainly supportive with in some cases correction of hypo-albuminemia with albumin infusions, and administration of diuretics is needed (Table 13.1).

Conclusion

MD is a rare condition in children of which the pathophysiology and etiology are not yet fully understood. New possible mechanisms and the involvement of genetics in the pathophysiology of MD have been suggested and are further investigated. Some viral, bacterial, and parasite infections are associated with the condition. The disease can only be diagnosed by gastroscopy and histology of gastric biopsies. The disease is self-limiting, and supportive therapy is advised.

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Viral Diarrhea

14

Alfredo Guarino and Eugenia Bruzzese

Epidemiology and Etiology

Although childhood diarrhea deaths have declined more than 80% from 1980 to 2015, diarrhea is still the second leading cause of death due to infections among children below 5 years of age worldwide after lower respiratory tract infections [1, 2], and it is estimated that almost 2 million children aged less than 5 years die each year in the world accounting for almost 20% of total child deaths. African and South-East Asia Regions account for 78% of all diarrhea deaths occurring among children in the developing world, and 73% of these deaths are concentrated in 15 developing countries [2, 3]. A too slow progressive reduction is observed and the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) estimated that in 2016, diarrhea was the fifth leading cause of death among children younger than 5 years with a rate of 70.6 deaths per 100.000. Diarrhea was responsible for 8.92% of all deaths in children younger than 5 years in 2016, with an estimated incidence of 1.75 episodes per child younger than 5 years. The number of diarrhea deaths among children younger than 5 years decreased by more than 50%, and also diarrhea incidence decreased between 2000 and 2016 [4]. Childhood wasting, unsafe water, and unsafe sanitation were the leading risk factors for diarrhea [4]. Table 14.1 shows mortality rates and incidence of diarrhea due to the main etiological agents.

Nevertheless, acute diarrhea is still a major problem in both developing and industrialized countries, but with two distinct consequences. In developing countries, enteric infections are highly common, and acute gastroenteritis is also responsible for a high mortality rate, whereas in industrialized countries, the incidence of diarrhea and the mortality is much lower than in poor countries, although not negligible. Gastroenteritis hospitalizations continue to require significant inpatient resources also in the United States; from 2010 to 2011 about 4/1000 children were hospitalized for acute gastroenteritis with an estimated direct cost of $200 million annually [5].

Table 14.1 Mortality rates and incidence of acute diarrhea in children under 5 years of age due to specific viral agents in 2016

	Deaths per 100.000 (95% UI)	Episodes per 1000 (95% UI)
Adenovirus	1·3 (0·8–1·8)	22·4 (16·0–30·1)
Norovirus	1.7 (0.8–3.1)	76.8 (30.1–159.6)
Rotavirus	20.3 (16.6–24.5)	408.6 (311.6–533.1)

Modified from GBD 2016 Diarrhoeal Disease Collaborators [4]

Rotavirus is the leading cause of acute gastroenteritis [4], and it is the most frequent agent of severe diarrhea in children <5 years of age, worldwide. Rotavirus infections affect virtually all children by the age of 5 years [6]. The Global Rotavirus Surveillance Network recently described the impact of Rotavirus on hospital admissions for acute gastroenteritis in children younger than 5 years in different countries with different vaccination programs. In countries that had not introduced specific vaccine in their national immunization programs, Rotavirus was detected in 38.0% of admissions for acute gastroenteritis annually whereas in those that have introduced the vaccine, Rotavirus was detected in 23.0%, showing a 39.6% relative decline following vaccine implementation [7]. Similar results have been recorded in Europe [8], where Rotavirus accounts for 55% of hospitalizations and one-third of emergency department visits due to community-acquired acute gastroenteritis in children aged <5 years. The burden of community-acquired Rotavirus gastroenteritis occurs in children aged <2 years with a peak in infants aged <6 months. It has a seasonal pattern, peaking in winter, coinciding with common respiratory infections. Every year, approximately 500,000 children younger than 5 years die because of diarrhea with Rotavirus being the most frequent pathogen in developing countries [7]. In industrialized countries, where there is generally good access to health care, mortality due to Rotavirus gastroenteritis is low.

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It has been estimated that in Europe approximately 6500 children died each year as a result of Rotavirus infection in the prevaccine era [9]. The mortality was due to severe and fast dehydration in previously healthy children and not only in children from identifiable risk groups. Recently, a total of 4161 stool specimens from children less than 5 years of age, hospitalized with acute gastroenteritis, were analyzed to investigate the specific etiology of diarrhea in Italy using real-time PCR technique. Rotavirus was the prevalent virus (24.7%) followed by Norovirus (19.6%), adenovirus (5.3%), and astrovirus (3%). Co-infections were detected in 8.3% of patients with viral gastroenteritis, and Rotavirus with Norovirus (70.6% of co-infections) or with astrovirus (9.6%) were the most common associations [10]. However, hospital admissions and deaths from Rotavirus gastroenteritis and all-cause acute gastroenteritis have declined following the progressive incorporation of Rotavirus vaccines into national immunization programs [7]. In the United States, before the introduction of specific vaccine in 2006, Rotavirus caused an estimated 20–60 deaths, 55,000–70,000 hospitalizations, 205,000–272,000 emergency department visits, and 410,000 outpatient visits each year [11]. After 10 years, hospitalizations and emergency department visits due to Rotavirus AGE were reduced by a median of 67% [12]. Rotavirus infection is therefore a major cause of hospitalization in children with acute gastroenteritis with substantial impact on healthcare resources and costs. Before Rotavirus vaccination programs the estimated average cost for an episode of diarrhea in inpatient was US$ 2300. Costs for acute diarrhea are not negligible, particularly if the so-called societal costs are considered. After Rotavirus vaccine introduction, the annual cost reductions ranged between $134 million and $257 million [13].

During the past four decades, there has been a dramatic increase in the number of newly recognized etiologic agents of gastroenteritis. Since 1970, more than 20 different microorganisms (viruses, bacteria, and parasites) have been recognized as etiological agents. Nevertheless, in up to 50% of acute gastroenteritis cases, the causative agent remains undiagnosed with traditional laboratory methods. This low diagnostic proportion stimulated research to use new tools such as broad-range PCR tests, pan-viral microarrays, and viral metagenomics for virus identification in patients with acute diarrhea. Although many viruses have been identified in fecal samples of patients with diarrhea, their causal relationship with diarrhea is largely unknown (Table 14.2).

In addition to Rotaviruses and Noroviruses, Apoviruses of the Caliciviridae, human Astroviruses of the Astroviridae family, and human enteric Adenoviruses of the Adenoviridae family are among the medically important viruses causing gastroenteritis in children. These agents are associated with outbreaks in semiclosed communities in all age groups and in immunocompromised patients and are able to induce severe, long-lasting diarrhea that occasionally may be fatal. The viruses most frequently responsible for acute gastroenteritis in children belong to four distinct families: Rotaviruses, caliciviruses, astroviruses, and enteric adenoviruses. Rotavirus and Norovirus are the two leading agents of acute diarrhea. Other viruses, such as toroviruses, picornaviruses (the Aichi virus), and enterovirus 22, play a minor epidemiological role. Finally, selected viruses induce diarrhea only in children at risk; these include Cytomegalovirus, Epstein–Barr virus, Picobirnaviruses, and HIV [14]. Very recently, a novel Coronavirus, SARS-COV-2 responsible for an acute respiratory syndrome, has been associated with gastrointestinal symptoms of acute gastroenteritis with vomiting and diarrhea being particularly frequent in pediatric patients [15].

Table 14.2 Role of different viruses in the etiology of acute diarrhea in children

Established	Probable	Possible in selected children or in outbreaks
Rotavirus	Torovirus	HIV Sapoviruses Caliciviruses
Norovirus	Aichi virus Enterovirus 22	Cytomegalovirus
Adenovirus		Epstein–Barr virus Picobirnavirus
Astrovirus	SARS-CoV-2	Coronavirus

Pathophysiology of Viral Diarrhea

In the classic and simple view, the pathogenesis of diarrhea may be divided into osmotic and secretory. Viral diarrhea was originally believed to be the consequence of endoluminal fluid accumulation osmotically driven by non-absorbed nutrients due to cell invasion and epithelial destruction by enteropathogenic agents. It is now known that several mechanisms are responsible for diarrhea, depending on the specific agents and the host features. In addition, selected viruses have multiple virulence pathways that act synergistically to induce diarrhea.

The mechanisms of diarrhea induced by group A Rotaviruses have been extensively investigated and provide a paradigm of the pathophysiology of viral diarrhea [16–18]. Rotavirus has both a tissue- and cell-specific tropisms, and it infects the mature enterocyte of the small intestine. The first step is virus binding to specific receptors located on the cell surface, the GM1 ganglioside. However, different Rotavirus strains bind in either a sialic-acid-dependent or sialic-acid-independent fashion. Most Rotaviruses, including all human strains, infect polarized enterocytes through both the apical and the basolateral sides, in a sialic-acid-independent man-

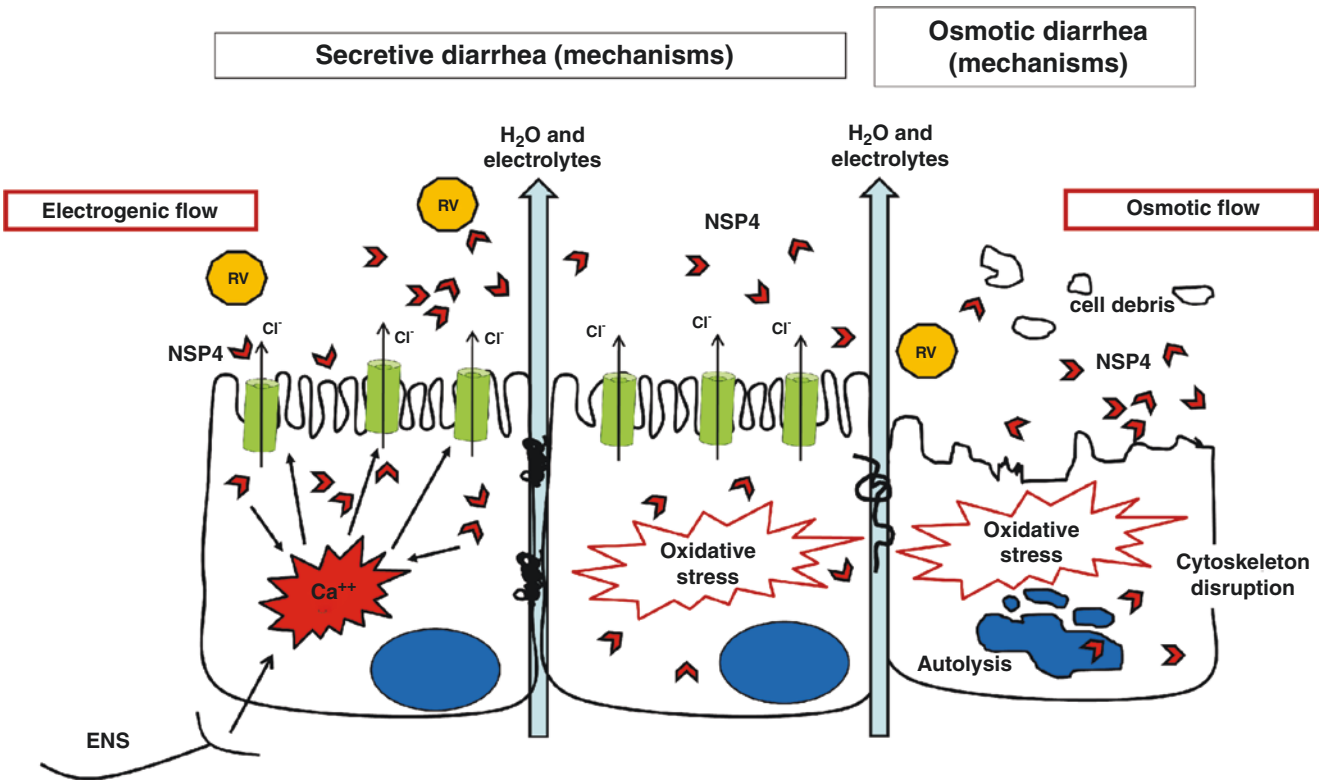

Fig. 14.1 Mechanisms of secretive and osmotic diarrhea induced by Rotavirus. During infection, Rotavirus interacts with enterocytes both as viral particles and through the NSP4 enterotoxin. In the first phase, NSP4 mobilizes intracellular calcium that, in turn, acts as the chloride channel CaCC. This event generates an electrogenic force which draws water and electrolytes into the intestinal lumen underlying the secretory diarrhea. Oxidative stress is an additional mechanism activated by NSP4 that affects secretory diarrhea. In the second phase, Rotavirus damages intestinal cells and epithelium resulting in an increase in intestinal permeability. Cellular debris, proteins, and other molecules released during cytotoxic damage generate an osmotic force underlying the osmotic diarrhea

ner, suggesting the presence of different receptors. The early stages of Rotavirus binding involve the viral protein (VP4) spike attachment and cleavage. After binding, Rotavirus enters into the cell by a multistep process that requires both VP7 and VP4 proteins. Infection of the villous enterocytes leads to cell lysis, thereby reducing the digestive–absorptive surface and not absorbed nutrients and driving fluids into the intestinal lumen through an osmotic mechanism (Fig. 14.1).

However, the destruction of villus-tip cells induces a compensatory proliferation of crypt cells. These immature enterocytes physiologically maintain a secretive tone, thus contributing to diarrhea with ion secretion, as the result of the imbalance between absorptive villous and secretory crypt cells. Thus, the cytopathic effect of Rotavirus results in both osmotic and secretory diarrhea. Histological changes induced by Rotavirus infection occur within 24 h of infection in animal models.

The enteric nervous system may also play a direct role in inducing fluid secretion, similar to that induced by cholera toxin and other intestinal secretagogues. The molecular mechanisms of fluid secretion have also been investigated. Rotavirus induces an increase in intracellular calcium levels, which is responsible for the disassembly of microvillar F-actin, the perturbation of cellular protein trafficking, the damage of tight junction, with a disruption of cell–cell interaction, and cytolysis. Rotavirus-induced diarrhea may be also associated with an increase in intestinal motility through the stimulation of myenteric nerve plexus [18]. In children with Rotavirus infection, the onset of diarrhea is abrupt and occurs in the absence of histological changes, suggesting that in the initial phase of the infection a secretory pathway is responsible for diarrhea. The identification of the nonstructural protein (NSP4), an enterotoxin produced by Rotavirus, responsible for fluid secretion but not for epithelial damage may explain this phenomenon. NSP4 is a multifunctional virulence factor (VF). It is released from infected cells and enters the cells through a specific receptor causing calcium-dependent chloride secretion. NSP4 also alters plasma membrane permeability and is cytotoxic. NSP4 is the only Rotavirus gene product capable of eliciting intracellular calcium mobilization. NSP4 also contributes to diarrheal pathogenesis by directly altering enterocyte actin distribution and paracellular permeability. Finally, NSP4 plays a role in the inhibition of the Na^+-dependent glucose transporter

Table 14.3 Mechanisms involved in Rotavirus-induced diarrhea

Mechanism	Effect
Enterocyte damage	Nutrient malabsorption/osmotic diarrhea
Crypt cell proliferation	Ion and water secretion/secretory diarrhea
NSP4 production	Increase in intracellular calcium, chloride secretion/secretory diarrhea
NSP4 inhibition of SGLT-1	Glucose malabsorption/osmotic diarrhea
Neuromediated vascular ischemia	Secretory diarrhea induced by neurotransmitter release
Inflammation	NF-kB, IL-8, Rantes release/osmotic, and secretory diarrhea
Stimulation of myenteric nerve plexus	Increase in intestinal motility

NSP4 nonstructural protein, *NF-κB* nuclear factor kappa B, *IL* interleukin, *SGLT-1* Na^+ -dependent glucose transporter

(SGLT-1). Glucose absorption as well as disaccharidase activities are impaired in Rotavirus enteritis, whereas the Na/amino acid co-transporters are not involved. Finally, Rotavirus diarrhea may also have an inflammatory component. The induction of cytokines is important in developing an inflammatory and immune response, especially in intestinal infection caused by bacteria. In Rotavirus infection, limited inflammation is detected by histological studies, suggesting that cytokines are effective in inducing a host immune response to Rotavirus diarrhea. However, it has been shown that Rotavirus-infected enterocytes activate nuclear factor kappa B (NF-κB) and the production of chemokines interleukin (IL)-8, Rantes, and growth-related oncogene (GRO)-a, of interferon (IFN)-α, and of granulocyte/macrophage–colony-stimulating factor (GM-CSF).

In conclusion, the primary target of Rotavirus is the enterocyte, which is induced to secrete fluids and electrolytes and is subsequently destroyed. On the other hand, the enterocyte acts as a sensor to the mucosa with the production of viral and endogenous factors and the activation of other cell types including neurons. Thus, Rotavirus-induced diarrhea is a multistep and multifactorial event, in which fluid secretion and cell damage are observed in a precise sequence, as shown in an intestinal cell line-based experimental model (Table 14.3) [17].

Clinical Signs and Symptoms of Viral Diarrhea

Usually, viral diarrhea has an abrupt onset and lasts 3–5 days. Generally, it is a benign condition with spontaneous recovery. In very few cases, diarrhea may be persistent lasting more than 7–15 days. Risk factors for persistent diarrhea in children are young age, early weaning, malnutrition, and immunodeficiency. In selected children, an acute onset may coincide with the first manifestation of celiac disease.

The predominant symptoms of acute gastroenteritis, regardless of the causative etiologic agent, are diarrhea and vomiting, associated or not with fever and abdominal pain. None of the symptoms that usually characterize Rotavirus-induced gastroenteritis has a strong predictive value. However, there are selected features that may help a differential diagnosis of viral versus bacterial infection [19, 20].

Children with viral intestinal infection generally have larger volumes of watery stools, suggesting small bowel involvement, and vomiting and fever are more frequent than in bacterial diarrhea. Each of these features contributes to dehydration. Another feature of viral gastroenteritis is its frequent association with respiratory symptoms due to the coinciding peak of common respiratory infections (Table 14.4).

In contrast, the presence of symptoms suggesting colitis, such as a high number of diarrheal episodes with small amount of stools, blood in the stools, high fever, and abdominal pain, is more likely associated with bacterial etiology (Table 14.4).

The severity of acute gastroenteritis is reflected by the degree of dehydration. This should be evaluated at first observation and during the follow-up to establish the efficacy of rehydration treatment. However, high persistent fever and lethargy suggest more severe clinical conditions and systemic involvement. Moreover, benign seizures, not related to electrolyte imbalances, have been reported for Rotavirus- and Norovirus-induced gastroenteritis [21, 22], but severe encephalopathies were reported in a recent surveillance study conducted in Germany on about 100 cases of severe diarrhea [23].

Clinical pattern of viral gastroenteritis is also associated with the specific pathogen (Table 14.5). When compared with other viral infections, Rotavirus infection is more frequently associated with high-grade fever (>38 °C), frequent diarrheal episodes (>7/day), and long-lasting diarrhea that results in significantly higher severity scores [24]. In contrast, children with Norovirus infection have significantly more episodes of vomiting than other viral infections, and in some cases, vomiting may be the only gastrointestinal symptom and up to 20% of children are present without diarrhea. However, Norovirus is only slightly less clinically severe than Rotavirus as agent of gastroenteritis in young children

Table 14.4 Clinical features associated with viral and bacterial agents of acute diarrhea in children

Viral diarrhea	Bacterial diarrhea
Watery diarrhea	High fever
High volume stools	Bloody stools
Vomiting	Dysentery
Fever	Abdominal pain
Presence of respiratory symptoms	Neurological signs

Table 14.5 Main clinical features of viral gastroenteritis related to specific etiologic agent

	Preferred age	Transmission route	Incubation	Symptoms		
				Diarrhea	Vomiting	Fever
Rotavirus	<5 years	Fecal–oral	1–3 days	+++	++	++
Norovirus	All ages	Fecal–oral, water, foods, environment	12 hours–2 days	++	+++	+
Adenovirus	<2 years	Fecal–oral	3–10 days	++	+	+

according to a systematic analysis. Intestinal infections due to adenovirus, on the other hand, seem to have milder clinical features [24].

Diagnosis

Most children with acute diarrhea have viral gastroenteritis. Microbiological examination is not helpful in the majority of cases and should be reserved for special circumstances. In fact, regardless of etiology, most children do not require any etiology-based treatment, and therefore identification of a specific pathogen is not generally needed. Microbiological investigation should, however, be performed during outbreaks, especially in childcare settings, schools, hospitals, or residential settings to identify the pathogen and establish its source in the attempt to reduce transmission. Stool samples should also be taken from children with bloody diarrhea, a history of recent foreign travel, and from young or immunocompromised children with high fever for whom antibiotic treatment is considered. Finally, it is also recommended to investigate children in whom diarrhea persists for more than 10–14 days, or when a noninfectious etiology for diarrhea is suspected, such as inflammatory bowel disease (IBD) (Table 14.6) [19].

Several techniques are available to identify the specific etiology of viral diarrhea. The gold standard is viral culture but its clinical application is limited, due to the costs, the delay in the results, and the complexity of the procedures. Immunofluorescence or latex agglutination is widely used to identify fecal viruses. Various molecular techniques offer easy, quick, and reliable diagnosis of viral gastroenteritis. In clinical laboratories, polymerase chain reaction (PCR)-based assays are considered as gold standard for the detection of viruses. Specific PCR is currently available for Norovirus, Rotavirus, adenovirus, cytomegalovirus, and other less common viruses. Among different techniques used to explore new viruses, such as conventional and next-generation sequencing, metagenomic has been a promising approach to study genetic material directly from samples and bypass the need for culturing the virus [25, 26]. Virome capture sequencing is another approach for viruses, in which millions of probes cover the entire genome of several viral taxonomies [27]. In Table 14.7, a list of main diagnostic methods for enteric viruses is presented. Diagnosis of viral gastroenteritis is performed detecting viral antigen or nucleic acid in fresh stool samples during the acute illness. Diagnosis of Rotavirus infection is generally made detecting Rotavirus antigen in stools using commercially available enzyme immunoassay (EIA) or latex particle agglutination test. Both are easy and inexpensive and have 90% sensitivity and 95% specificity. Other methods for Rotavirus detection include electron microscopy, viral isolation, and RNA polyacrylamide gel electrophoresis. Reverse transcription–polymerase chain reaction (RT-PCR) is not used in clinical practice because the test has too high sensitivity, and it may detect virus when it is not causing disease [28]. Real-time RT-PCR is the preferred laboratory method for detecting Noroviruses. This assay has high sensitivity, is able to detect less than 10 Norovirus copies, and allows a semiquantitative estimation of viral load. The assay is generally performed on stools, but it can also be used for other biological samples such as vomitus, foods, water, and environmental specimens mainly for outbreak evaluations.

Table 14.6 Indications to microbiological evaluation in children with acute diarrhea

Condition
Age < 3 months
Shock or septic appearance
>10 liquid stools/day, high fever, dysentery, bloody stools
Recent history of travel
Immunocompromised children
Outbreak
Protracted or chronic diarrhea

Table 14.7 Diagnostic tools for enteric viruses' identification

Diagnostic assay	Specific technique	Etiologic agent
Cell culture		Rotavirus
Electron microscopy		Rotavirus
Serological	ELISA, Bioluminescent EIA	Rotavirus, Astrovirus, Adenovirus
Nucleic acid based	PCR variants (Multiplex RT-PCR, Real-Time RT-qPCR, Immuno-PCR) isothermal amplification	Norovirus
Next-generation methods	Mass spectrometry, NGS, Microarray	Sapoviruses of the Caliciviridae, Human Astroviruses of the Astroviridae

Differential diagnosis of viral gastroenteritis may include food poisoning, which may be eventually indirectly related to microbial etiology. Food poisoning has a more rapid onset, often with vomiting and is more common in children than in infants, being related to ingestion of at-risk foods. On the other hand, when diarrhea is persistent, lasting more than 7–10 days, a different etiology should be considered [29]. Common causes of persistent diarrhea include small intestinal bacterial overgrowth, lactose intolerance, and cow's milk protein intolerance. In addition, chronic inflammatory conditions of the small and/or large intestine such as celiac disease or IBD may have an acute onset or be triggered by viral enteritides.

Specific Viruses

Rotavirus

Rotavirus is a double-stranded RNA virus belonging to the Reoviridae family. The virion, 70–75 nm, is composed of a three-layered protein capsid that encompasses 11 distinct segments of genomic RNA, each coding for a different capsid or nonstructural protein. The internal core contains VP 1, 2, and 3; the inner capsid contains VP4; the two outer capsid proteins encoded by genes 4 and 7, namely VP4 and VP7, represent the only established neutralization antigens of the virus. The protective role of antibodies directed against these proteins has been confirmed in both experimental animal models and humans. A possible role has been suggested for antibodies directed at the inner capsid protein VP6, which is not associated with in vitro neutralization. The nonstructural proteins NSP1, NSP2, and NSP4 are VFs in mice. Rotavirus groups A–F have been described, but only groups A, B, and C have been identified in humans. Most human infections are caused by group A Rotaviruses that are classified into serotypes by a dual classification system based on neutralizing antigens on two outer capsid proteins, VP7 (G serotype) and VP4 (P serotype). To date, 14 G serotypes and 11 P serotypes have been identified in humans. There is substantial genetic diversity within each G- and P-type. Predominant serotypes vary from year to year and from region to region. G1P is the globally predominant strain, representing over 70% of Rotavirus infections in North America, Europe, and Australia. G9 strains now constitute the predominant strains in some parts of Asia and Africa, and G8 strains are proportionally more frequent in Africa [30–32]. In South America, G5 strains have emerged in children with diarrhea, and G9 is associated with more severe disease in Latin America [33].

Specific strains may express stronger VFs, which could be related to the severity of symptoms. More severe presentations may also be related to the reintroduction of strains in areas where they have been previously absent. The epidemiology of Rotavirus shows a link with cold seasons with a higher incidence during fall and winter.

Transmission occurs by fecal–oral route, both through close person-to-person contact and by fomites. Viruses are shed in high concentrations in the stool of Rotavirus-infected persons. Children shed large numbers of viruses in stool, during the acute illness but they may shed Rotavirus 2 days before and up to 10 days after the onset of symptoms. The virus may also be transmitted by respiratory droplets. Spread within families, institutions, hospitals, and childcare settings is common. Rotavirus is a major cause of acute gastroenteritis in children attending childcare. Rotavirus is also responsible for nosocomial infection during the winter with an incidence as higher as 3% hospitalization in a meta-analysis of 20 studies in Europe and North America [34], prolonging hospital stays and increasing medical costs [35, 36].

The incidence of nosocomial infections is directly related to the duration of hospital stay, and the incidence of nosocomial Rotavirus-induced gastroenteritis may be as high as 8–10/1000 child-days of hospitalization.

The incubation period for Rotavirus diarrhea is short, usually less than 48 h. Rotavirus is able to determine a large spectrum of disease, ranging from asymptomatic shedding to severe dehydration, seizures, and even death. Rotavirus gastroenteritis typically begins with acute onset of fever and vomiting followed 24–48 h later by watery diarrhea Symptoms generally persist for 3–8 days, although protracted episodes have been reported occasionally. Fever is usually of low grade and occurs in up to half of all infected children. Vomiting occurs in 80–90% of infected children, and it is usually brief, lasting 24 h or less in most children. Dehydration and electrolyte disturbances are the major complications of Rotavirus infection and occur most often in the youngest children. In hospitalized children, gastroenteritis associated with Rotavirus is more severe than in cases in which Rotavirus was not detected, with more severe dehydration, higher incidences of vomiting and parenteral rehydration. Children with immunodeficiency, particularly those with T-cell immunodeficiencies or severe combined immunodeficiency (SCID), and children after bone marrow transplantation are at higher risk of severe and prolonged diarrhea and central nervous system complications.

Norovirus

Noroviruses are single-stranded RNA viruses belonging to the family *Caliciviridae*. The prototype virus of the Noroviruses, Norwalk virus, was identified in 1972. The availability of molecular diagnostic methods based on reverse transcription-PCR (RT-PCR) highlighted the etiologic role of Norovirus in epidemic and sporadic gastroenteritis [37]. Norovirus is now a well-documented leading

agent of epidemic gastroenteritis in all age groups, causing >90% of nonbacterial and ≈ 50% of all-cause epidemic gastroenteritis worldwide [38]. The impact of Norovirus disease may be much greater than previously thought, and the disease may be more severe in selected populations.

The Norovirus genome comprises a single-stranded, positive-sense, polyadenylated RNA of approximately 7.5 kb in length, encompassing three open reading frames (ORFs 1–3).

Noroviruses encompass five distinct genogroups (GI–GV) with GI, GII, and GIV infecting humans. The Norovirus genogroups are further divided into genotypes and variants (subgenotypes) based on the sequence diversity.

The mechanisms of Norovirus-induced diarrhea are not well defined. Many viral factors can interfere with basic cellular functions. Several observations showed that, in infected mucosa, villus length decreased by 25%, whereas crypt length was unchanged. Villus blunting and the shortening of the villi observed in this viral infection depend on the Norovirus infection of intestinal epithelial cells located on the apical area of villi. Infected cells undergo an increase in cell death rates with a reduction of the overall absorptive surface.

Evaluations of human intestinal biopsies from Norovirus-infected showed an active Cl^- secretion, consistent with cystic fibrosis transmembrane conductance regulator (CFTR) activation. A reduction of occludin expression as well as claudins 4 and 5 corresponding with a marked decrease in transepithelial resistance has also been observed. In addition, Norovirus produces two proteins, p48 and p20. The former interferes with cell proteins involved in the regulation of vesicle trafficking, whereas p20 impairs actin cytoskeleton structure leading to intestinal barrier dysfunction.

Intestinal mucosal immunity is also affected by Norovirus. Dendritic cells were depleted in Norovirus-infected mucosa associated with an altered antibody response but dendritic cells were required for a dissemination of the virus to secondary lymphoid tissues supporting the idea that enteric viruses can use dendritic cells to facilitate their dissemination within the host [39]. In addition, VF1 expression enables Norovirus to establish efficient infection interfering with interferon-mediated response pathways and apoptosis [40, 41]. Finally, gut microbiota is highly altered in patients with Norovirus gastroenteritis resulting in a low grade of diversity and increased proteobacteria [42]. Elevated proteobacteria is a common feature in patients with dysbiosis, and a reduction in the diversity is associated with several altered functions of gut microbiota [43].

Norovirus gastroenteritis may occur in three distinct epidemiological settings, and is associated with a broad spectrum of clinical outcomes. First, foodborne gastroenteritis generally affects large numbers of healthy adults over a short time period, with symptoms typically resolving within 1–3 days Second, healthcare-associated infection, which occurs in semiclosed settings such as hospital wards and residential/care homes, can be very challenging to contain. Elderly and compromised individuals are frequently affected and are at high risk of prolonged clinical course, typically 4–6 days, with a not negligible mortality rate. However, its shedding persists in immunocompromised subjects. Third, Norovirus gastroenteritis may occur sporadically in children, where Norovirus is the second most common cause of acute viral gastroenteritis after Rotavirus, often requiring hospitalization. Compared with Rotavirus enteritis, the duration of vomiting and diarrhea is significantly longer but Norovirus infection is slightly less severe in terms of severity score and need of intravenous (IV) rehydration compared to Rotavirus infection. In different geographical areas, Norovirus is more prevalent in winter. In the United States, Noroviruses cause 20 million episodes of gastroenteritis every year and are increasingly recognized as a cause of severe disease, leading to more than 900 deaths and substantial rates of hospitalization, major losses for outbreaks, productivity losses missed school/workdays. The total cost is estimated to be as high as 10.6 billion based on an estimate of almost 70/1000 persons per year [44, 45]. Of those estimates slightly less than half are related to children, where Norovirus has become the major pathogen in post Rotavirus vaccine era [46].

SARS-CoV-2 Virus

A novel Coronavirus, named SARS-CoV-2, has been responsible for a viral pandemic with hundreds of millions of infections and more than 5 millions of deaths. The coronavirus disease 2019 (COVID-19), has a spectrum of clinical manifestations ranging from fever, dry cough, and dyspnea to pneumonia, acute respiratory distress syndrome, and multiple organ failures. Less frequently, patients with SARS-CoV-2 infection present nausea, vomiting, and diarrhea. Diarrhea is a frequent symptom in coronavirus infections; it was detected in up to 30% of patients with MERS-CoV and 10.6% of patients with SARS-CoV infection [47]. Similarly, data from early reports of the current pandemic suggest that gastrointestinal symptoms are not uncommon in patients with COVID-19 [48, 49]. A cohort study of 140 COVID-19 patients showed that gastrointestinal symptoms were observed in almost 40% of the patients, including nausea (17%), diarrhea (13%), and vomiting (5%) [50]. Atypical cases of SARS-CoV-2 infection characterized by gastrointestinal symptoms were reported [51]. Clinical studies show an incidence of diarrhea ranging from 2% to 50% of cases [15], and the frequency of diarrhea and vomiting was higher in newborns and infants than in adults [15]. The majority of evidences simply described the presence of diarrhea in patients affected by COVID19 without a specific character-

ization in terms of number of evacuations, stool consistency, and duration of symptoms. SARS-Cov-2 was found in the stools, and viral RNA shedding in stool was detectable for longer period than in nasopharyngeal swabs raising the hypothesis of fecal–oral route as a transmission pathway. There are many hypotheses to explain why COVID-19 causes digestive symptoms but the exact molecular mechanism needs to be further investigated. SARS-CoV-2 and SARS-CoV showed a high genomic homology indicating that the new virus could bind ACE2 and infect humans. Structural studies showed that the new SARS-CoV-2 not only binds ACE2, but its binding affinity for human ACE2 is 10–20 times stronger than its 2003 SARS-CoV predecessor. Second, SARS-CoV-2 indirectly damages the digestive system through a chain of inflammatory responses. Another possible factor might be antibiotic-associated diarrhea; patients with more severe COVID19 frequently received a cocktail of antibiotics and antiviral drugs, responsible for changes in the composition and function of digestive tract flora [52]. Several case series describing the clinical presentation of COVID19 in adults showed that patients without digestive symptoms were more likely to be cured and discharged than patients with digestive symptoms. This could be due to either viral replication in the gastrointestinal tract causing more severe disease or that patients who do not initially have typical respiratory symptoms present with later stages of disease [48]. No data on the efficacy of antidiarrheal drugs are available, but an adequate rehydration and potassium monitoring should be performed as in all patients with diarrhea. Scattered data indicate that these symptoms disappeared after antiviral therapy (oral lopinavir and ritonavir), supporting the link between symptom and COVID-19 disease.

Evaluation and Treatment of Children with Acute Diarrhea

The initial clinical approach to the child with acute gastroenteritis is clinical evaluation. Hydration status in children should be assessed based on easily observed signs and symptoms. Dehydration should be estimated using a score system. An easy to use and reliable score system to evaluate dehydration in children is the Clinical Dehydration Scale (CDS) [53]. It consists of four clinical items: general appearance, eyes, mucous membranes, and tears, each of which is scored 0, 1, or 2 to a total score of 0–8 (Fig.14.2).

Severe dehydration is an indication for hospital admission and for IV rehydration. Indications to hospital admission are reported in Table 14.8.

Specific antiviral treatment is generally not needed to treat Rotavirus infection. The current treatment of acute Rotavirus gastroenteritis consists of oral rehydration solution (ORS) and early introduction of feedings, like any other form of acute diarrhea [19]. Adequate fluid and electrolyte replacement and maintenance are the key to managing viral gastroenteritis. Oral rehydration is the preferred method unless the child has intractable vomiting that would require IV rehydration. Children who are mildly or moderately dehydrated should receive 50–100 ml/kg of ORS over 4 h and should be reevaluated often for changes in hydration status.

Clinical item	0	1	2
General appearance	Normal	Thirsty, irritable or lethargic	Drowsy,cold or sweaty; comatose or not
Eyes	Normal	Slightly sunken	Very sunken
Mucous membranes	Normal	Sticky	Dry
Tears	Normal	Decreased	Absent

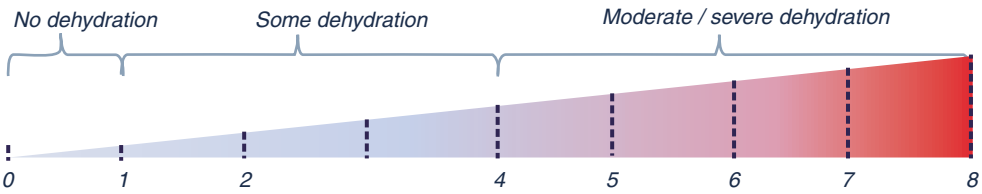

Fig. 14.2 Clinical dehydration scale for children with acute diarrhea

Table 14.8 Indications to hospital admission in children with acute diarrhea

Age < 3 months
Shock
Severe dehydration (>9% of body weight)
Neurological abnormalities (lethargy, seizures)
Intractable or bilious vomiting
Failure of oral rehydration
Suspected surgical condition
Caregivers cannot provide adequate care at home and/or there are social or logistical concerns

Reduced osmolarity ORS (50/60 mmol/L of Na) is the first-line treatment for children with acute gastroenteritis. Reduced osmolarity ORS is more effective than full-strength ORS (75/90 mmol/L of Na) as measured by key clinical outcome indicators as stool output, vomiting, and need for supplemental IV therapy. The so-called ESPGHAN (the European Society for Pediatric Gastroenterology Hepatology and Nutrition) solution, containing 60 mmol/L of Na, may be used in children with acute gastroenteritis as it has been used successfully in several randomized clinical trials (RCTs) and in a number of non-RCTs. However, several data including a recent in vitro study challenged the optimal composition of ORS [54].

The second step of treatment of acute diarrhea, in children with mild-to-moderate dehydration, is early resumption of feeding after rehydration therapy. It is recommended that (1) breastfeeding should be continued throughout rehydration; (2) the usual age-appropriate diet should be started immediately after initial rehydration (4–6 h); (3) dilution of formula or use of a modified milk formula is usually unnecessary. To continue feeding is important for limiting the nutritional consequences of decreased intake and for stimulating digestion and absorption of essential nutrients during diarrheal illness. A systematic review showed that lactose-free diet in children who are not breastfed may result in shorter duration of diarrhea. However, most of the trials included in the review were from children hospitalized in developed countries [55]. In children in low- and middle-income countries, where the double burden of diarrhea and malnutrition is greatest, the use of locally available age-appropriate foods should be promoted for the majority of acute diarrhea cases particularly if the access to milk formulas and specific expensive ingredients is limited [56]. In persistent diarrhea, withdrawal of lactose should be considered.

Enteral or intravenous (IV) rehydration is needed in selected conditions and is usually applied in children in hospital. The former is effective and has no side effects and should be preferred. Children who are severely dehydrated with changes in vital signs or mental status require emergency IV rehydration. For children presenting with shock, rapid IV infusion of isotonic crystalloid solution (0.9% saline or lactate Ringer's solution) with 20 ml/kg bolus should be used. For children with severe dehydration without shock, rapid IV rehydration with 20 ml/kg/h of 0.9% saline solution for 2–4 h is indicated. A dextrose-containing solution may be used for maintenance [57] .

There is solid and compelling data demonstrating the efficacy of selected probiotics as an adjunct to rehydration therapy in reducing the intensity and duration of symptoms. The guidelines of ESPGHAN [19] and the Universal Guidelines [58] for the management of acute gastroenteritis in children recommend the use of two different probiotics, *Lactobacillus rhamnosus* strain GG (LGG) and *Saccharomyces boulardii* in adjunct to the ORS for the treatment of acute diarrhea in children [59, 60]. Both *Lactobacillus* GG and *S. boulardii* reduce the duration of diarrhea by approximately 1 day. *Lactobacillus* GG is particularly effective in Rotavirus-induced diarrhea. In developed countries, *Lactobacillus* GG given in a daily dose of 10 billion colony-forming units (CFU) per day has proven effective in reducing the risk of protracted diarrhea and the duration of hospitalization in Rotavirus gastroenteritis [61, 62] although a recent large size high-quality study found no evidence of efficacy in North American children in emergency department setting [63].

Other pharmacological interventions have been studied in the attempt to reduce the duration of acute diarrhea and for some there is proof of efficacy. Diosmectite can be considered in the management of acute gastroenteritis as it is effective in reducing the duration of diarrhea [19]. A Cochrane systematic review concludes that smectite used as an adjuvant to rehydration therapy may reduce the duration of diarrhea in children with acute gastrointestinal infections, may reduce stool output, but has no effect on hospitalization rates or need for intravenous rehydration [64].

A recent meta-analysis assessed the efficacy of Racecadotril as an adjunct to ORS compared with ORS alone or with placebo. Compared with placebo, Racecadotril significantly reduced the duration of diarrhea and the need for IV rehydration [65]. Recently, a Cochrane systematic review evaluating trials conducted in children between 3 months and 5 years, both outpatient and inpatient from France, Spain, Peru, India, Kenya, and Ecuador, showed that Racecadotril is a safe drug but has limited benefit in improving acute diarrhea. The beneficial effect of Racecadotril is to reduce the risk of rehydration failure. No sufficient data are available to evaluate the effect on duration of diarrhea, number of stools, and length of hospital stay [66].

In Table 14.9, the active intervention for acute gastroenteritis is reported.

Diarrhea treatment (ORS, zinc, antibiotics for dysentery, and management of persistent diarrhea) and use of Rotavirus vaccine were responsible for nearly 50% of the reduction of diarrhea mortality from 1980 to 2015. Improvements in

Table 14.9 Interventions to be considered for active treatment of viral diarrhea

	Lactobacillus rhamnosus strain GG (LGG)	*Saccharomyces boulardii*	Smectite	Racecadotril
Stool volumes reduction	+	+	+	+
Antimicrobial effect	+	–	–	–
Single oral dose	–	–	–	–
Side effect	–	–	Constipation	Headache, vomiting, and constipation
Freely available	Not everywhere	Not everywhere	Not everywhere	Not everywhere
Level of evidence	Meta-analysis	Meta-analysis	Meta-analysis	Systematic review
Setting	In/outpatients	In/outpatients	In/outpatients	Inpatients
Cost	Low	Low	Low	Low
Palatability	++	±	–	+

nutrition and improvements in water, sanitation, and handwashing were even more important interventions effective in reducing the burden of infectious diarrhea. To further minimize the diarrhea-related mortality, all these interventions should be scaled up [67].

Because vomiting may make difficult or impossible oral rehydration, ondansetron may be used in single dose to overcome the peak phase and allow ORS administration. Although loaded with an FDA and EMA warning for potential cardiac severe effects, it is often used in emergency settings to reduce admission related to the need for IV rehydration [68].

Management of Viral Diarrhea in Immunocompromised Children

Management of acute gastroenteritis in children generally does not require microbiological investigation because acute infectious diarrhea is a self-limited disease and its treatment is independent of the etiology so that the identification of specific pathogen is not needed. Only in selected conditions and situations, it may be necessary to investigate the cause of diarrhea in order to establish a specific etiologic treatment. Immunocompromised children, mainly children with chronic diseases treated with immunosuppressive and immunomodulatory medications, are at high risk of infectious diarrhea. Viruses like Rotavirus, Norovirus, sapovirus, and adenovirus are the most common agents of infectious diarrhea in immunocompromised children and are responsible for severe prolonged disease course and complications like hypovolemia, dehydration, electrolyte imbalance, and malabsorption. Despite an etiologic treatment is possible for acute bacterial diarrhea, no specific drug is available for treatment of viral diarrhea. The oral administration of human serum immunoglobulins to treat Rotavirus- and Norovirus-induced diarrhea in immunocompromised patients has been previously reported [69, 70]. Recently a retrospective cohort review assesses the efficacy of oral immunoglobulin (OIG) in treating hospitalized immunocompromised children with diarrhea. Nineteen children immunocompromised for solid organ transplant, malignancy, and inflammatory bowel diseases were treated with oral immunoglobulins 100–300 mg/kg/day for 2–5 days. Eighteen of 19 children (95%) showed a significant reduction of stool output within 10 days of initiation of therapy; a complete symptom resolution was documented in 42% of patients [71].

Vaccines for Viral Diarrhea

Enteric viral infections are still a leading cause of mortality and morbidity in young children in both low- and middle-income countries, and in immunocompromised patients. Vaccination is an effective preventive approach against the fecal–oral transmitted infection particularly in resource-limited countries where sanitation systems and supply of safe drinking water are not easily achievable. Developing effective vaccines for enteric pathogens continues to be very challenging also because of a limited understanding or characterization of immune correlates to protection. Vaccine-induced IgG antibody responses are often measured, but assessment of IgA particularly mucosal IgA responses should be emphasized. The greatest challenge in the development of new vaccines is represented by the heterogeneity among genotypes or serotypes of viral pathogens. Vaccines are available only for few enteric pathogens including Rotavirus and cholera. No vaccines are available for most other enteric viral and bacterial pathogens responsible for acute gastroenteritis. Despite a progressive decrease in diarrhea-related deaths, Rotavirus is still a major cause of mortality mainly in developing countries. Rotavirus disease is prevented by vaccination, but there are cultural, organizational, and financial barriers to the implementation of immunization, the main being that families, physicians, and policy-makers wrongly believe that Rotavirus diarrhea is not

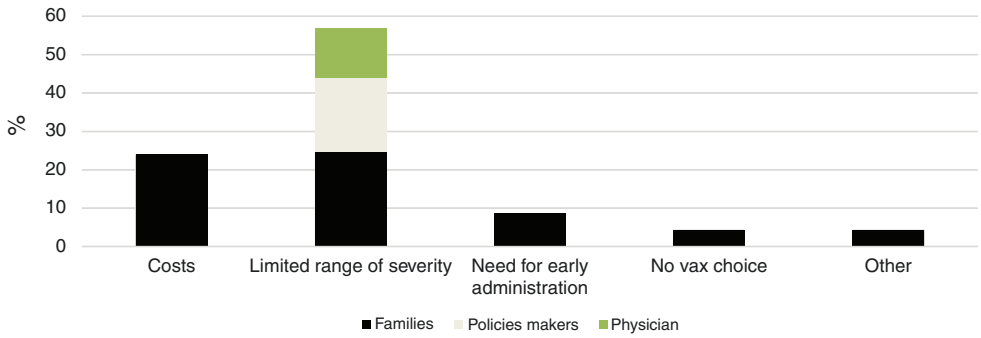

Fig. 14.3 Major barriers toward immunization against Rotavirus. (Modified from: Lo Vecchio et al. [75])

a severe disease (Fig. 14.3). WHO and other authorities recommend universal immunization and consider it a priority in countries with high Rotavirus gastroenteritis-associated deaths, such as in south and southeastern Asia and sub-Saharan regions. Vaccine development strategies are based on live-attenuated Rotavirus vaccines that can be administered by the oral route. The goal for a Rotavirus vaccine is to replicate the degree of protection against the disease induced by the first natural infection that occurs in infants. Therefore, the primary outcome of a vaccine program is the prevention of moderate to severe disease. Rotavirus vaccines decrease the number of children admitted to the hospital with dehydration and decrease the burden of office visits or telephone calls due to Rotavirus gastroenteritis.

At present, there are several vaccines two of which were distinctly developed. The first is a live-attenuated oral monovalent Rotavirus vaccine derived from a G1P8 human Rotavirus strain attenuated through serial passages in cell culture. It provides 85% protection against severe rotaviral gastroenteritis and 100% protection against the most severe dehydrating rotaviral gastroenteritis episodes [72]. The other vaccine is a pentavalent reassortant oral vaccine derived from a G6P [5] bovine Rotavirus strain and common human Rotavirus strains carrying G1, G2, G3, G4, and P [8] human Rotavirus surface proteins. It has a three-dose schedule with the first dose within 6 weeks of age and the last by 24 weeks of age. Its efficacy against Rotavirus gastroenteritis of any severity was 74% and against severe Rotavirus gastroenteritis was 98% [73].

In 2007, the WHO recommended inclusion of Rotavirus vaccine in the immunization programs of Europe and America and, in 2009, expanded the recommendation to all infants over the age of 32 weeks worldwide. Other qualified institutions and agencies included Rotavirus universal immunization as a major priority to reduce the mortality for acute gastroenteritis [19].

The burden of Rotavirus disease in the United States and elsewhere has been reduced significantly since the introduction of Rotavirus vaccines.

Data from eight high-income and middle-income countries showed a 49–89% reduction of Rotavirus-associated hospital admissions and a 17–55% of all-cause gastroenteritis-associated hospital admissions among children younger than 5 years, within 2 years of vaccination policy. Hospitalization rates were reduced from 60 to 93% depending on vaccine coverage, age groups, and Rotavirus season. Studies have also evaluated the impact of Rotavirus vaccination on all-cause gastroenteritis or diarrhea-related hospitalizations, emergency visits, and outpatient or physician office visits, and consistent reductions were seen for all these parameters. Effective Rotavirus vaccines are most needed in resource-poor countries, where mortality associated with Rotavirus is high. Although Rotavirus vaccines resulted less effective in developing countries than developed countries (50–64% vs. 85–98%), the burden of disease prevented by vaccination is greater in developing countries, probably due to the greater baseline incidence of severe Rotavirus disease. Initial results from African countries that have implemented routine Rotavirus vaccination supported the efficacy of Rotavirus vaccines. There are several hypotheses to explain the difference in the efficacy of Rotavirus vaccination between developing and developed countries. Poor nutritional status, enteric co-infections, or the interference of anti-Rotavirus antibodies in breast milk may hamper live virus vaccines, which are more common in children leaving in resource-poor countries. Even considering a lower efficacy, the use of live Rotavirus vaccines should be implemented in these countries to reduce the high mortality due to Rotavirus gastroenteritis. Overall, the live-attenuated Rotavirus vaccine has been substantially effective in reducing the burden of Rotavirus infection [74]. Although the effectiveness of the rotavirus vaccine is widely demonstrated, its implementation is still low; barriers to implementation vary in relation to setting but elevated cost of vaccine, limited perception of RV illness severity by the families, public-health authorities or physicians and the timing of administration are still the major barriers to large-scale Rotavirus vaccination programs (Fig. 14.3) [75]. Implementation of Rotavirus vaccine has been included in the priorities of interventions against childhood mortality [76].

Unlike Rotavirus for which vaccines have been licensed and recommended, there is no vaccine licensed for the

single-stranded RNA Norovirus which now is the most common viral cause of gastroenteritis in developed countries where Rotavirus vaccines have been introduced [77]. In contrast to Rotavirus which exclusively infects children, Norovirus causes gastroenteritis in people of all ages mostly among young children and the elderly over 65 years. The typical clinical outcome of Norovirus infection is acute gastroenteritis but it can cause also severe dehydration and death if infection progresses without intervention and treatments. The high disease burden caused by Norovirus particularly associated with foodborne disease and outbreaks and the potential severe outcomes in both children and elderly people has led to develop vaccines for the prevention of Norovirus disease.

In conclusion, despite major achievements in reducing death rates particularly in children in deprived settings, viral gastroenteritis remains a huge problem that needs urgent interventions. In addition also in rich countries, viral diarrhea should not be neglected as it is responsible for economic and health losses and for a total burden of death that probably exceeds that induced by diseases that are certainly more severe but also much more rare. This translates into a risk of death that is reversed in the general population of children when compared to children that are affected by a more severe but also much less rare disease.

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Bacterial Infections of the Small and Large Intestine

15

Rachel Bernard and Maribeth Nicholson

Introduction

Diarrhea is the result of an imbalance of ion and water movement through the intestinal epithelium. In normal conditions, the intestine absorbs 8–9 L of fluid while only 100–200 mL are excreted in the stools daily [1–3]. In addition, the intestine absorbs nutrients while simultaneously forming a selective barrier to dangerous substances and pathogenic bacteria. Finally, the intestine is colonized with a wide community of bacteria that exert many beneficial functions, collectively known as the intestinal microbiome [4]. Resident commensal and foreign bacteria interact intimately with the gut epithelium and affect host cellular and innate immune responses [5]. Enteric pathogens may hamper the physiological handling of electrolytes and water transport processes by secreting toxins that disturb the function and/or the structure of the intestinal epithelium [6].

A number of cellular and molecular mechanisms are implicated in infectious diarrhea induced by bacteria, viruses, and parasites. Pathogen-specific virulence factors affect a wide range of cell functions such as ion absorption and secretion, barrier function, and membrane-trafficking pathways causing fluid accumulation in the intestinal lumen [7, 8].

Several bacterial enterotoxins are able to increase chloride (Cl−) secretion and to reduce sodium (Na+) absorption by acting on apical membrane-located transporters or on the lateral spaces between cells, regulated by tight junctions (TJs) [9–11]; other pathogens induce cell damage by targeting the cytoskeletal network which is directly implicated in paracellular fluid absorption [12, 13]. Invasive pathogens cause an inflammatory diarrhea characterized by an increase in polymorphonuclear cells (PMNs) in the lamina propria leading to an excess of cytokine secretion and activation of the enteric nervous system via neuropeptides, eventually resulting in dysenteric diarrhea. Furthermore, a specific class of *Escherichia coli*, the enteropathogenic *E. coli* (EPEC), induces diarrhea through damage of the apical enterocytes, thereby reducing the intestinal absorptive surface [14]. Regardless of the mechanism, diarrhea is the manifestation of an altered movement of ions and water that follows an osmotic gradient. The pathophysiology of diarrhea is summarized in Fig. 15.1.

Intestinal Ion Transport and Barrier Functions

In normal conditions, fluid transport across the intestinal epithelial cells is a finely balanced process between fluid absorption and fluid secretion. Dysregulation of the normal intestinal ion transport and barrier functions leads to diarrhea. Transporters, pumps, and ion channels mediate the flow of solutes through the cell membrane and water follows passively. Absorption function of the apical enterocytes depends on both the electroneutral and the electrogenic cotransporters and exchangers [15]. Major ion transporters involved in electrolyte absorption include the sodium–hydrogen exchanger (Na+, H− exchanger or NHE), the chloride–bicarbonate (Cl−, $HCO3^-$) exchanger, and the epithelial sodium channel (ENaCs) [16]. There are several NHEs positioned on the apical membrane of small intestinal villus and surface epithelial cells in the colon that play a major role in Na+ and water absorption [17]. NHE1 is located on the basolateral surface of the enterocyte, whereas NHE2 and NHE3 have an apical localization [18, 19]. The relative contribution of NHE2 and NHE3 depends on their localization within the intestine; NHE3 is the main mediator of electroneutral Na+ uptake [20]. The electrogenic ion absorption is the second major source of apical ion absorption through specific channels. The ENaC is the most prominent apical Na+ channel in

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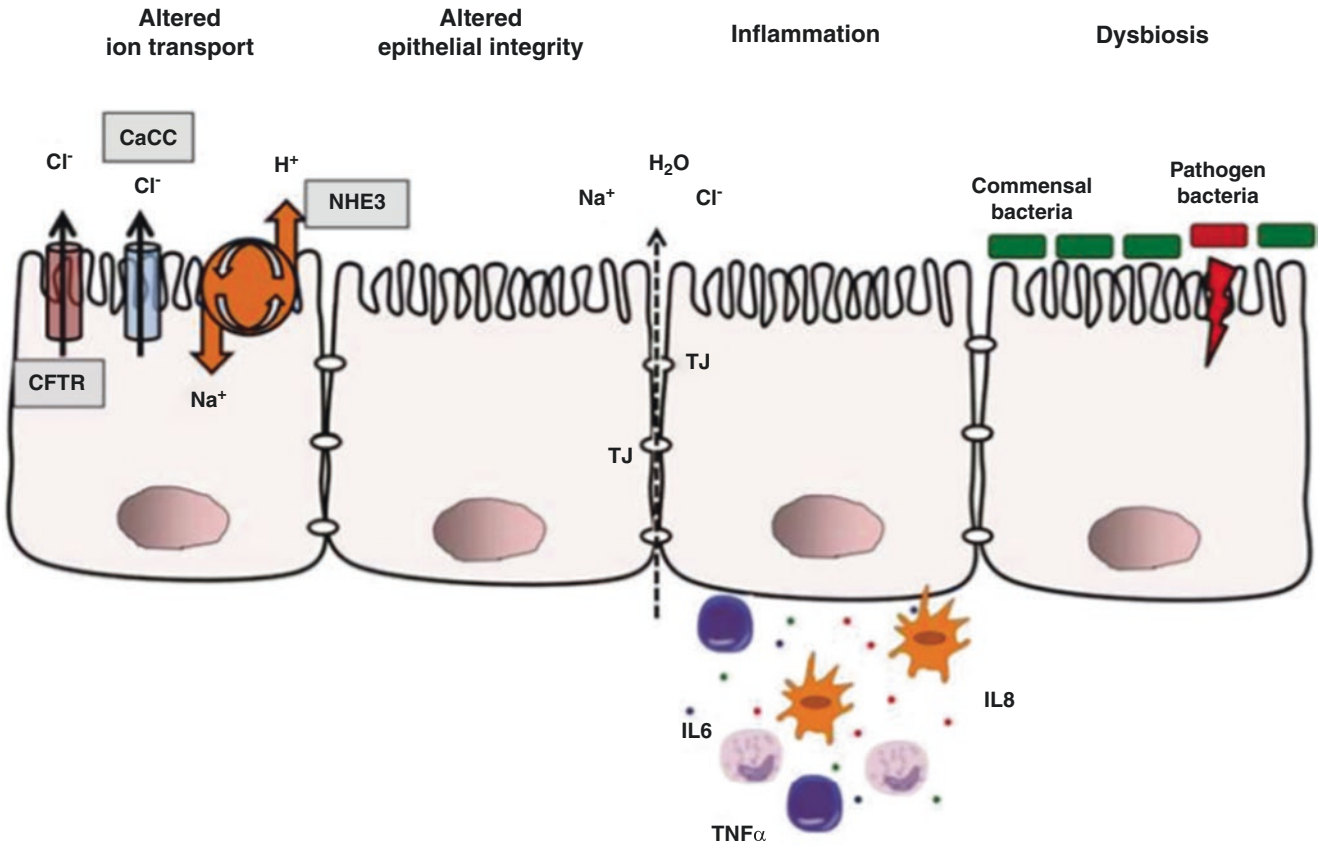

Fig. 15.1 General mechanisms causing diarrhea. Bacterial toxins can affect intestinal epithelial functions through different mechanisms. Chloride secretion and water secretion depend on the altered functionality of CFTR, CaCC, and NHE channels. Alterations in TJ structures alter the electric gradient causing the movement of both ions and water. Pathogen bacteria induce inflammation through the recruitment of immunity cells and the upregulation of pro-inflammatory cytokines. Finally, an altered gut microbiome composition (dysbiosis) supports pathogen infections. *CFTR* cystic fibrosis transmembrane conductance regulator, *CaCC* calcium-activated chloride channels, *NHE* Na^+, H^+-exchanger, *TNFα* tumor necrosis factor-α. *IL* interleukin

the colon and allows Na+ influx into the cell along its electrochemical gradient [16].

A basal level of fluid secretion is necessary for accomplishing the nutrient digestive functions and is mediated by three main transporters comprising the cystic fibrosis transmembrane conductance regulator (CFTR), the Na+/K+/2Cl cotransporter type 1 (NKCC1), and calcium-activated chloride channel (CaCC) transporter [21]. In the intestine, water secretion is a passive process driven by active ion secretion, predominantly by Cl-secretion. The CFTR channel is located on the brush border and is regulated by cyclic AMP (cAMP) and cyclic guanosine monophosphate (cGMP) [22]. cAMP and cGMP are stimulated by secretagogues, and the channel is responsible for chloride efflux and thus water secretion in basal conditions [3]. Initially, chloride is taken up across the basolateral membrane via NKCC1, which facilitates the uptake of Na+, K+, and 2Cl-ions across the basolateral membrane, and thereby supplies chloride for secretion via an electroneutral process [23]. Chloride accumulation is a passive process driven by the Na+ concentration gradient which is maintained by the basolateral Na+/K+-ATPase [24]. Two distinct potassium channels are located in the basolateral membrane allowing for potassium recycling, thus preventing cellular depolarization and ultimately preserving the electrical driving force for chloride exit from the cell. Therefore, chloride accumulates until apical chloride channels are opened. The bulk of chloride output occurs via the cAMP-dependent CFTR chloride channel [25]. However, there is an additional class of chloride channels, the CaCC, that are expressed in the enterocyte apical membrane [21]. These channels drive chloride secretion in response to agonists of cytosolic calcium [21].

The most common mechanism of bacterial diarrhea is the release of bacterial toxins. Bacterial enterotoxins activate signaling molecules such as cAMP, cGMP, or intracellular Ca2+, which, in turn, opens cellular Cl– channels leading to an increase in Cl– secretion and consequently of water secretion [26]. The intestinal epithelium forms a selective barrier between the lumen (the external environment) and the body. The electrochemical gradients also depend on TJs that

provide a barrier necessary for an efficient transcellular transport [27]. The TJs also support a polarized distribution of membrane proteins on the basal and apical compartments of epithelial cells. The plasma membranes of adjacent intestinal epithelial cells are linked through the TJs, where claudins, zonula occludens 1 (ZO1), occludin, and F-actin interact. E-cadherin, alpha-catenin 1, beta-catenin, catenin delta1, and F-actin interact to form the adherens junction [28]. The loss of barrier function, as a consequence of TJ disruption, impairs the vectorial absorption and secretion by the intestinal epithelium. During an infection, the redistribution of apical and basal proteins provides new attachment sites for bacteria. Several bacterial toxins act on the TJ protein functions inducing an epithelial damage. At least four toxins produced by *Clostridium spp*. and a toxin produced by selected *E. coli* strains have been demonstrated to affect intestinal TJs [29, 30].

In this complex scenario, different bacterial species, each with one or more virulence factors, induce diarrhea in a specific mode. This includes the interaction with a specific receptor, the release of virulence factors such as adhesion molecules or enterotoxins, and a cascade of events occurring within the enterocyte.

Bacterial Diarrhea

Vibrio cholerae

This comma-shaped, gram-negative rod is easily identified in the stool using gram stain and is responsible for cholera, a potentially fatal acute diarrheal disease in humans. Although cholera is now rare in developed countries, it remains a major cause of diarrheal morbidity and mortality in many developing countries [31].

V. cholerae is a diverse species with both pathogenic and non-pathogenic variants. Only cholera toxin-producing strains are associated with the clinical development of cholera. Serogroups *V. cholerae* O1 and O139 have caused widespread epidemics, although only *V. cholerae* O1 remains a current cause of global outbreaks [32]. Other toxin-producing serogroups of *V. cholerae* have been associated with small outbreaks of cholera, and non-toxin-producing strains can cause a cholera-like illness associated with gastroenteritis and sepsis. The infection is transmitted by the fecal–oral route and is spread through feces-contaminated food and water. Virtually, all cases of non-O1 *V. cholerae* infections in the United States are associated with eating raw shellfish. The period of incubation ranges from a few hours to 5 days [33, 34].

V. cholerae possess a single flagellum and is highly motile, which enables it to swim through mucus and arrive at the mucosal surface [35]. During infection, *V. cholerae* secretes several toxins, but the most important of these is cholera toxin (CT), an enterotoxin that causes profuse diarrhea when it colonizes the small intestine. CT consists of a single copy of the A subunit and five copies of the B subunit. The B subunit binds to the plasma membrane, while the A subunit activates adenylate cyclase resulting in elevated cAMP production. The production of cAMP leads to secretion of chloride via the CFTR domain [36, 37]. Na+ absorption is also decreased through the apical sodium transporters, NHE2 and NHE3 [38]. Overall, this leads to an increase in NaCl levels in the intestinal lumen and drives water by osmotic force.

In addition to CT, *V. cholerae* produces several other toxins that contribute to the enterotoxicity of this pathogen by modulating ion secretion and altering barrier function. The toxins that directly affect ion secretion include the accessory CT (ACE), which stimulates Ca2+-dependent Cl− secretion and the non-agglutinable *V. cholerae* heat-stable enterotoxin (NAG-ST), which activates guanylyl cyclase, thus stimulating cGMP production and leading to protein kinase G (PKG)-mediated activation of CFTR. *V. cholerae* cytolysin (VCC) creates anion permeable pores into the cell wall [39], and zonula occludens toxin (Zot) interacts with tight junctions affecting the paracellular pathway [40].

Symptoms range from asymptomatic colonization to severe diarrhea associated with dehydration and shock. Vomiting is frequent, although fever less common. Stools are classically described as “rice water” due to the presence of mucus in clear stools but are frequently without associated tenesmus. Profuse watery diarrhea and vomiting may lead to significant fluid and electrolyte losses that can occur at a rate of 1 L/hour, causing a massive dehydration which can occur within 12 hours of symptom onset. Although mild symptoms may be clinically indistinguishable from other causes of gastroenteritis, the profound loss of fluids and dehydration are unique to severe cholera [41].

As with many other infectious diarrheal diseases, the mainstay of therapy for *V. cholerae* is aggressive rehydration, which can be done orally in the absence of severe volume depletion or shock. Intravenous fluids should be urgently administered in those with severe hypovolemia, preferable with Lactated Ringer’s [42, 43]. In general, there are no long-term complications of cholera when it is appropriately treated. Electrolyte abnormalities, pneumonia potentially from aspiration in the setting of vomiting, and shock have been described and associated with mortality [44].

Antibiotics (e.g., macrolides, tetracyclines, and fluoroquinolones) are an adjunctive therapy for patients with severe volume depletion and may be of particular use in epidemic settings as they decrease the duration of shedding. Antibiotic therapy is recommended for patients with severe dehydration secondary to *V. cholerae* gastroenteritis since it reduces the duration of diarrhea by approximately 50% and bacterial

shedding by about 3 days [45]. Overall, most guidelines updated in the last decade recommend single-dose azithromycin as the preferred first-line therapy for children and doxycycline as first-line therapy for cholera in adults [46]. Ciprofloxacin can be used for susceptible strains. Antibiotic therapy is usually not needed for gastroenteritis caused by non-cholera *Vibrio spp*. Currently, there are two internationally licensed oral cholera vaccines used in areas with endemic cholera and during cholera outbreaks [47].

Salmonella

Salmonellae are motile gram-negative bacilli that cause several clinical infections in humans, including gastroenteritis, enteric fever, bacteremia and endovascular infection, and focal metastatic infections such as osteomyelitis or abscess [48]. Although there are many types of *Salmonella*, they are frequently divided into two main categories: those that produce typhoid and enteric fever and those that induce gastroenteritis.

Salmonella Typhi and *Salmonella* Paratyphi colonize only humans and cause enteric fever or typhoid fever, a systemic illness characterized by high-grade fever, gastrointestinal symptoms, and occasional neurologic manifestations. Infections are acquired through close personal contact or through the ingestion of water or food contaminated with human feces. Typhoid fever continues to represent a global health problem, with more than 11–17 million cases/year [49]. Chills, headache, cough, weakness, and muscle pain are frequent prodromes, and most symptoms resolve within 4 weeks without antimicrobial treatment [50]. Gastrointestinal perforation, due to necrosis of Peyer's patches in the bowel wall, is a known complication seen in week 2 and 3 of the disease but occurs more commonly in adults than children [51].

In contrast, infections with nontyphoidal *Salmonella* are increasing in developed countries and are frequently isolated from the stool of patients with gastroenteritis. In the United States, nontyphoidal salmonellosis is one of the leading causes of foodborne disease, and *Salmonella* Enteritidis and *Salmonella* Typhimurium are among the serotypes most frequently isolated. *Salmonella* Enteritidis is the leading bacterial cause of foodborne diarrheal outbreaks in the United States, with eggs and contaminated raw fruits and vegetables identified as major vehicles [52].

Salmonella gastroenteritis is most commonly associated with ingestion of poultry, eggs, and milk products, as well as contact with pets, including reptiles and live poultry. Symptoms of *Salmonella* gastroenteritis typically occur within 8–72 hours following exposure and are clinically indistinguishable from gastroenteritis caused by many other pathogens. Among children, the diarrhea can progress to grossly bloody stools [53]. Severe extra-intestinal infections can include life-threatening sepsis and focal infections in the meninges, bones, and lungs. Nontyphoidal *Salmonella* gastroenteritis is usually self-limited. Fever generally resolves within 2–3 days, and diarrhea within 4–10 days [54].

Salmonella are pathogenic through their ability to invade, replicate, and survive in human host cells, resulting in an inflammatory ileocolitis. *Salmonella* enter into the submucosal lymphoid system through the ileum and colon by selectively using fimbriae to adhere and attach to specialized epithelial cells overlying Peyer's patches known as M (microfold) cells [55]. Sa*lmonella* pathogenesis includes plasmid-encoded virulence factors that encode proteins vital for bacterial spread and invasion, survival within macrophages after phagocytosis, and signaling to neutrophils, causing cell destruction [56]. Additionally, the *Salmonella* Pathogenicity Islands (SPIs) encode multiple virulence factors, including Type III secretions systems (TTSS) which deliver a variety of effectors into intestinal epithelial cells [56]. Enterotoxins may also play a role in *Salmonella* gastroenteritis as well as the production of signaling molecules which trigger fluid secretion and diarrhea in the host [57]. Overall, microbial invasion of the mucosa results in an acute inflammatory reaction with disruption of the epithelial barrier, resulting in the presence of mucus, red blood cells, and polymorphonuclear leukocytes in the stool. The bacteria can also disseminate from the intestines to cause systemic disease.

Antibiotics should not be used in an otherwise healthy child with *Salmonella* gastroenteritis as they have not demonstrated efficacy and do not prevent complications. A systematic Cochrane Review showed that antibiotic therapy of *Salmonella* gastroenteritis did not significantly affect the duration of fever or diarrhea in otherwise healthy children or adults compared to placebo or no treatment [58]. In addition, adverse treatment events include prolonging fecal excretion of *Salmonella* and antimicrobial resistance [58]. Antimicrobial therapy is indicated in the setting of severe disease and in patients at higher risk for complications or invasive disease, including children younger than 3 months of age or those with chronic gastrointestinal tract disease, malignant neoplasms, hemoglobinopathies, HIV infection, or other immuno-suppressive illnesses or therapies [59] (Table 15.1). Bloody diarrhea does not necessarily indicate the need for antibiotic treatment. Secondary *Salmonella* bacteremia, with potential extra-intestinal focal infections, occurs more often in children with certain underlying conditions, and in neonates or young infants. Antibiotic therapy with azithromycin is suggested for well-appearing patients. Alternatively, ampicillin or trimethoprim–sulfamethoxazole may be considered for susceptible strains, once susceptibilities are available [59]. Fluoroquinolones are an additional option [59, 60].

Table 15.1 Bacterial infections of the gastrointestinal tract

Pathogen	Signs and symptoms	Transmission	Incubation	Antibiotic indications
Vibrio cholerae	Profuse watery diarrhea and vomiting, potentially leading to severe dehydration	Contaminated food or water	Hours–5 days	Severe dehydration
Salmonella	Watery diarrhea, dysentery Extra-intestinal infections include sepsis and focal infections in the meninges, bones, and lungs	1. Contaminated food such as eggs, poultry, milk products 2. Contact with contaminated animals such as reptiles	8–72 hours	High-risk children: infants less than 3 months of age and children with underlying hemoglobin disorders or immune deficiency
Clostridioides difficile	Watery diarrhea, dysentery, fever, and abdominal pain Septicemia, pseudomembranous colitis, and perforation can occur in severe cases	Ingestion of spore in susceptible host	2–3 days	Symptomatic patients (> or equal to 3 unformed stools in 24 hours)
Shigella	Watery diarrhea, dysentery, fever, malaise, anorexia, occasional vomiting, and tenesmus Complications include HUS, seizures, rectal prolapse, intestinal obstruction, and colonic perforation	Contaminated food or water	3 days	1. High-risk children; immunocompromised patients and children with severe toxemia or suspected bacteremia 2. Culture-proven and symptomatic patients in daycare, institutions, or hospitalized children 3. Children with suspected shigellosis during an outbreak setting or with a positive culture in a household or daycare contact
Campylobacter	Watery diarrhea, dysentery, vomiting Complications including Guillain–Barre syndrome and reactive arthritis	1. Contaminated water or food such as unpasteurized milk or poultry 2. Direct contact with animals or animal products	3–6 days	Dysenteric form and to reduce transmission in daycare centers and institutions
Yersinia	Prolonged watery diarrhea, dysentery, fever, vomiting, and abdominal pain Exudative pharyngitis and cervical adenitis may also be present	Contaminated water or food including undercooked pork	4–6 days	Severe dehydration
Escherichia coli[a]				
ETEC	Watery diarrhea, abdominal cramps, and vomiting Occasional fever	Contaminated food or water	10–72 hours	Severe dehydration[a]
EPEC	Watery diarrhea, fever, abdominal cramps, and vomiting Can be severe in infants	Contaminated food or water	9–12 hours	Severe dehydration[a]
EHEC	Crampy abdominal pain, vomiting, and watery diarrhea are the first symptoms; can progress to dysentery Fever absent or low grade Possible severe complications such as HUS	1. Contaminated food or water 2. Direct contact with infected animals	3–4 days	Severe dehydration[a]
DAEC	Watery diarrhea rarely associated with vomiting or abdominal pain	Contaminated food or water	Unknown	Severe dehydration[a]
EAEC	Prolonged watery, mucoid diarrheal illness with low-grade fever, and little or no vomiting	Contaminated food or water	8–48 hours	Severe dehydration[a]
EIEC	Watery diarrhea, dysentery	Contaminated food or water	4–7 days	Severe dehydration[a]

[a]Treatment recommendations for non-STEC-confirmed *E. coli*

Clostridioides (Formerly *Clostridium*) *difficile*

Clostridioides difficile is a gram-positive toxin-producing anaerobe that exists in a highly resistant spore form in the environment. *C. difficile* infection (CDI) is now recognized as the most common cause of antibiotic-associated diarrhea and the leading cause of gastroenteritis-associated death in the United States with approximately 12,800 deaths in 2017, although incidence rates vary dramatically worldwide [61].

Increasing incidence of CDI has been demonstrated in both adults and children. Data from 22 freestanding children's hospitals demonstrated a rise in the incidence of *C. difficile* infection (CDI) from 2.6 to 4 cases per 1000 admissions from 2001 to 2006 [62]. An additional study from a national database of hospitalized children reported a doubling of the incidence rates of CDI from 2003 to 2012 [63]. Increasing incidence is likely multifactorial, including the emergence of virulent strains (NAP1/BI/027), the increased use of antibiotics, and alterations in diagnostic testing methods, such as the transition to highly sensitive nucleic acid amplification-based testing (NAAT).

The pathogenesis of *C. difficile* largely depends on the altered balance of the intestinal microbiota, allowing pathogenic strains of *C. difficile* to infect the intestine [64]. Outside of the colon, *C. difficile* exits in spores which are highly heat, acid, and antibiotic resistant. If ingested and able to colonize the intestine, they convert to a vegetative toxin-producing form. *C. difficile* disease activity is related to the production of one or more toxins: toxin A (TcdA), toxin B (TcdB), and the *C. difficile* transferase toxin (CDT) [65]. Whereas TcdA and TcdB are considered the primary virulence factors, CDT may increase the severity of CDI in certain clinical strains. After undergoing receptor-mediated endocytosis, TcdA and TcdB disrupt the epithelial tight junctions and induce epithelial cell death, causing direct injury to the colonic epithelium. In addition, the toxins stimulate epithelial cells to release proinflammatory cytokines and neutrophil chemoattractants, which lead to the acute inflammatory response which is characteristic of CDI [66]. This disruption of the epithelial barrier and intense inflammatory response with associated tissue damage is thought to contribute to the formation of pseudomembranes, a manifestation of severe CDI. Alternatively, CDT intoxication leads to loss of the actin-based cytoskeleton of the host cell wall, formation of microtubule-based cell protrusions that increase pathogen adherence, and enhanced production of inflammatory cytokines [66].

Antibiotic use continues to be the most widely recognized risk factor for CDI, although additional risk factors have been identified in children including the presence of comorbidities such as malignancy and inflammatory bowel disease and the use of acid suppression [67]. However, CDI can occur in the absence of antibiotic history and in a large case–control study of pediatric patients, >40% had no antibiotic use in the 12 weeks prior to CDI diagnosis [68].

C. difficile can cause a range of disease manifestations, from asymptomatic colonization or mild diarrhea to severe CDI complicated by toxic megacolon, hypotension, shock, and death. Asymptomatic colonization is more common in infants and children <3 years of age, although the mechanisms for this remain unclear. In healthy infants younger than 1 month of age, *C. difficile* has been recovered from an average of 37% of the infant's stools, with a range of 25–80% of infants harboring *C. difficile* as a harmless commensal. This rate continues to decline until age one year when the rate is ~10% in healthy infants [69–71]. Symptomatic disease is less common in infants and young children, although it has been described. Asymptomatic colonization has also been described in children with medical comorbidities. Children with cancer, cystic fibrosis, and inflammatory bowel disease have asymptomatic colonization prevalence of 30%, 50%, and 17%, respectively [72–74]. There are significant difficulties differentiating CDI from *C. difficile* colonization in these patients.

For most patients, symptomatic CDI begins several days or weeks after antibiotic therapy. Acute onset and mild-to-moderate watery diarrhea is the most common clinical feature. Fever and abdominal pain are common, and gross blood is occasionally apparent [75]. Severe and fulminant CDI is less common in children than adults, but recurrent CDI occurs in approximately 12–30% of pediatric patients [76–78]. Recurrent CDI is the return of CDI after initial symptom resolution and generally occurs 1–2 months after the initial infection. Risk factors in children include malignancy, community-acquired CDI, receipt of concomitant antibiotics, and the presence of a tracheostomy tube [76, 77, 79].

The diagnosis of CDI relies on a combination of clinical and laboratory features and can be challenging in the pediatric patient. Current laboratory testing for *C. difficile* detection relies on the detection of *C. difficile* toxin and/or toxigenic *C. difficile* organism and cannot differentiate colonization from CDI. Most commonly, testing is performed by nucleic acid amplification-based testing (NAAT) alone or as part of a multistep algorithm including enzyme immunoassay for glutamate dehydrogenase (GDH) or toxin [80]. Toxigenic culture and cell culture cytotoxicity assays are rarely performed in clinical settings due to slow turnaround times and need for technical expertise. Testing for CDI should be performed on symptomatic children with acute onset diarrhea (≥3 unformed bowel movements per 24 hours) with clinical features suggestive of CDI or known risk factors. Testing should be limited in children <3 years of age unless there is a high suspicion for CDI [81].

Discontinuing the inciting antibiotic, when able, is an important step in CDI management. An initial episode of mild-to-moderate CDI in a pediatric patient can be treated

with either oral metronidazole (30 mg/kg per day orally in four divided doses) or oral vancomycin (40 mg/kg per day in four divided doses) (Table 15.1) [80]. Although randomized controlled trials in adults have found vancomycin to be more efficacious for CDI than metronidazole [82, 83], prospective studies in children are lacking. Oral vancomycin is the preferred agent for children with severe or fulminant infection. In those unable to tolerate oral therapy, rectal vancomycin and IV metronidazole can be considered. Recurrent CDI can be treated with a second course of the same antibiotic in the setting of a first recurrence. The optimal management of subsequent recurrences is not well established but may include the use of oral vancomycin given in a pulsed-tapered fashion or fidaxomicin [80]. Alternative therapies include the use of rifaximin, nitazoxanide, intravenous immunoglobulin, and fecal microbiota transplantation (FMT). In a study of 372 pediatric patients with CDI who underwent FMT, 81% had a successful outcome with no recurrence of CDI at 2 months post-FMT [84]. Additional study in children is warranted.

Shigella

Shigella is a gram-negative, non-lactose-fermenting, nonmotile bacillus that causes 88 million cases of diarrhea and approximately 164 associated deaths worldwide per year [85]. There are four species of *Shigella*: *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D). *Shigella sonnei* is the main species type in developed countries, and outbreaks occur predominantly in institutions such as daycare centers. In developing countries, *S. flexneri* and *S. dysenteriae* predominate and most cases are transmitted by fecal–oral spread and contamination of common food and water sources [86]. *S. dysenteriae* is the most dangerous *Shigella* species due to its production of Shiga toxin, an enterotoxin that induces intestinal secretion of solutes and water.

Shigella is highly contagious and requires a significantly lower inoculum to causes disease than many other bacterial enteritidis [87]. All four species of *Shigella* are invasive and cause an inflammatory diarrhea. The cellular responses to various steps of the invasion process are the primary cause of the inflammation. *Shigella* strains cross the colonic enterocytes through M-cells (cells that overlie mucosal lymphoid follicles) by using a type III secretion system (T3SS) protein [88]. The T3SS injects bacterial proteins into the host cell that activate host cytoskeletal signaling pathways. *Shigella*-induced macropinocytosis occurs through extensive rearrangements of the host cell cytoskeleton. *Shigella* also has strategies to escape the entry vacuole, move freely in the host cytosol, replicate in the host's intracellular compartment, and spread from cell to cell [89]. The destruction of macrophages after emergence from M-cells causes an initial release of IL-1β, which attracts polymorphonuclear leukocytes (PMNs). PMNs amplify inflammation and can cause many of the symptoms of the disease. *Shigella* also disrupts tight junction proteins including claudin-1, ZO-1, ZO-2, and dephosphorylates occludin [90].

After an incubation period averaging 3 days, *Shigella* gastroenteritis classically begins with fever, malaise, anorexia, and occasional vomiting. Initial watery diarrhea can progress to dysentery (bloody diarrhea) within hours to days. Small-volume mucoid stools with blood are present in approximately 50% of children with Shigellosis [91]. In an immunocompetent host, symptoms are generally self-limited and last no more than 7 days. Rare manifestations may occur, including hemolytic uremic syndrome (HUS) in children, thrombotic thrombocytopenic purpura (TTP) in adults, seizures, reactive arthritis, rectal prolapse, intestinal obstruction, and colonic perforation. Neonates and children with an underlying immunodeficiency are at higher risk of complications [92].

Diagnosis is made via stool culture, although the presence of white blood cells and red blood cells on microscopy is suggestive [93, 94]. Molecular testing platforms using polymerase chain reaction (PCR) are also appropriate, although do not allow for susceptibility testing. In the absence of specific antibiotic treatment, children with *Shigella* gastroenteritis shed the organism for up to 4 weeks; children with immune deficiency shed for much longer, even in the absence of symptoms.

The goals of antibiotic therapy for *Shigella* include cure and hastening eradication of the organism from the feces. Several controlled studies have shown that appropriate antibiotic treatment of *Shigella* gastroenteritis significantly reduces duration of fever, diarrhea, and fecal excretion of the pathogen, and thus infectivity [95–97]. This has a large impact on transmissible populations such as children attending daycare centers and those admitted to hospitals or other institutions. Antibiotic treatment may also reduce the complications including the risk of HUS after *S. dysenteriae* infection [98].

However, antimicrobial therapy is not recommended for most children with mild disease or spontaneous recovery. Empiric antimicrobial therapy for shigellosis in children is recommended in high-risk individuals such as immunocompromised patients and children with severe toxemia or suspected bacteremia (signs of suspected bacteremia include leukocytosis, hypothermia, temperature >39 °C [102.2 °F], and lethargy) after blood and stool cultures have been collected (Table 15.1) [59]. Ill patients in daycare, institutions, or children that are hospitalized with symptomatic culture-proven *Shigella* should also be treated. Antibiotics may also be warranted in children with suspected shigellosis during an outbreak setting, or with a positive culture in a household or daycare contact [99, 100].

Antibiotic susceptibility testing is essential for management of patients with suspected *Shigella* infection [101]. Worldwide, antimicrobial resistance is an increasing problem, and patterns of resistance vary geographically. Oral azithromycin and parenteral ceftriaxone are the suggested first-line treatments of shigellosis in the United States in children <18 years of age if the antibiotic susceptibility of the isolate is unknown [102]. Trimethoprim–sulfamethoxazole or ampicillin should be used only if the isolated strain is known to be susceptible given the high worldwide resistance [103]. Parenteral therapy is indicated for children who cannot tolerate oral medications and have immunodeficiency, or patients with bacteremia. Intravenous ceftriaxone for 5 days is the first-line parenteral therapy. Ciprofloxacin for 5 days is an alternative to ceftriaxone if there is no other safe or effective option. The WHO guidelines recommend ciprofloxacin as first-line treatment and pivmecillinam, azithromycin, or ceftriaxone as second-line therapy [104]. Persistent fever, bloody stools, or stool frequency after 3 days of therapy may be a sign of treatment failure [105].

Campylobacter

Campylobacter are small, spiral-shaped gram-negative bacilli [106]. *Campylobacter* enteritis is typically caused by *Campylobacter jejuni* or *Campylobacter coli* which are common commensals in the intestinal tracts of animals. Approximately, 90% of infections are due to *C. jejuni* in most regions, although the clinical symptoms related to *C. jejuni* or *C. coli* are indistinguishable [107]. *Campylobacter* enteritis is transmitted by the fecal–oral route via direct contact with animals or animal products such as poultry and unpasteurized milk or via contaminated water [108, 109]. Most *Campylobacter* infections are transmitted through the preparation and/or consumption of chicken. Person-to-person transmission is uncommon.

After 3–6 days of incubation, typical gastroenteritis symptoms begin abruptly with vomiting and profuse diarrhea. In most children, diarrhea lasts 4–5 days and is usually mild and self-limited. Bloody stools may be present in more than half of children and mimic intussusception, and severe abdominal pain, when present, may uniquely resemble acute appendicitis [110]. Ultrasound can be helpful in differentiating bacterial ileocecitis from acute appendicitis. Fever can occur but is less common in older children [111]. Acutely, *Campylobacter* enteritis can be associated with cholecystitis, peritonitis, rash, and myocarditis or pericarditis. Late-onset complications include reactive arthritis, similar to what follows other diarrheal infections, and Guillain–Barre syndrome (GBS) [112, 113]. Between 30% and 40% of GBS is attributable to *Campylobacter* infection and neurologic symptoms generally develop 1–2 weeks after infection [114].

Campylobacter's spiral shape and long polar flagella promotes motility and chemotaxis enabling the organism to invade and colonize the intestinal tract [115, 116]. Adhesion and attachment of the organism are multifaceted and fimbriae-like filaments, and adhesion-related molecules aid in this process [117–119]. A high-molecular-weight plasmid, pVir, has been identified in some clinical Campylobacter isolates and has been shown to enhance the invasive capabilities of *C. jejuni* [120, 121]. Pathogenic *C. jejuni* strains also produce cytolethal distending toxin (CDT), a nuclease which may play a role in suppressing innate immunity by inducing death of macrophages and inducing a local inflammatory response [122, 123].

The diagnosis of *Campylobacter* is established by stool culture or culture-independent techniques, such as nucleic acid amplification tests (NAAT) or polymerase chain reaction (PCR). Supportive therapy including hydration and the correction of fluid and electrolyte losses is the mainstay of treatment in children. Antibiotic therapy for *Campylobacter* gastroenteritis is recommended mainly for the dysenteric form (bloody stools, high fever) and to reduce transmission in daycare centers and institutions (Table 15.1) [124]. In most locations, the first-line drug is azithromycin, but the choice should be based on local resistance patterns [59]. A meta-analysis of 11 double-blind, placebo-controlled trials showed that antibiotic treatment of gastroenteritis caused by *Campylobacter* spp. reduces the duration of intestinal symptoms by 1.3 days [125]. The effect was more pronounced if treatment was started within 3 days of illness onset and in children with *Campylobacter*-induced dysentery.

Yersinia

Yersinia species are gram-negative coccobacilli and facultative anaerobes. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are two important human enteropathogens that cause yersiniosis and are widely distributed in the environment, with swine as the major reservoir [126]. Infection is transmitted predominantly through the fecal–oral route; yersiniosis is often caused by the consumption of undercooked pork products or contaminated drinking water [127].

Y. enterocolitica uniquely passes through the stomach epithelial cells and localizes in lymphoid tissue and regional mesenteric lymph nodes [128]. This invasion process is due in part to *Yersinia's* elaborate mechanisms to buffer the gastric acidity, such as producing urease that releases ammonia from urea and provides relative acid resistance [129]. The process of adhesion and invasion mainly comprises the proteins encoded by three genes, *yadA*, *invA*, and *ail* [130]. Yersinia also expresses many virulence factors which are temperature dependent and aid in organism survival in different environments [131]. Both chromosomal and plasmid-

derived virulence factors play a role in *Yersinia* pathogenesis. The virulence of *Y. enterocolitica* strains mostly depends on the presence of the plasmid of *Yersinia* virulence (pYV), the most known and important virulence marker of *Y. enterocolitica* [132]. Another important immune evasion mechanism of *Y. enterocolitica* includes the injection of *Yersinia* outer membrane proteins (Yops) into host cells by T3SS [133]. Additionally, enterotoxin Yst is one of the most significant factors leading to diarrhea in yersiniosis [134].

The incubation period is 4–6 days with foodborne transmission. Acute yersiniosis is characterized by fever, vomiting, abdominal pain, and watery diarrhea, which may contain blood [135, 136]. In children, high fever and vomiting are common, with abdominal pain observed less frequently. Accompanying presenting symptoms include exudative pharyngitis and cervical adenitis, which are unique to *Yersinia* and less commonly associated with other bacterial enteritidis. Sore throat was reported by up to 20% of patients in some reports [137, 138].

The onset of symptoms can be more subacute, while the duration of diarrhea can be longer than for other causes of acute bacterial gastroenteritis [139]. Diarrhea typically lasts for 14–22 days, but fecal excretion may persist for 7 weeks or longer. Both abdominal complications and extra-intestinal complications are uncommon. Pseudoappendicitis has been described as acute yersiniosis can present with right lower abdominal pain, fever, and vomiting [140]. Bacteremic spread may rarely result in abscess formation and granulomatous lesions in the liver, spleen, lungs, kidneys, and bone, as well as meningitis and septic arthritis. The most common post-infectious sequelae are erythema nodosum, reactive arthritis, uveitis, and Reiter's syndrome [141]. Stool culture is the preferred test for patients with intestinal symptoms. In the setting of pharyngitis or septicemia, throat and blood cultures may be diagnostic as well. *Yersinia* can also be cultured from surgical specimens such as the appendix or mesenteric lymph nodes [142].

Antibiotic treatment is not warranted in most cases of acute yersiniosis, and limited data are available regarding antibiotic efficacy [143]. Parenteral therapy with third-generation cephalosporins or oral ciprofloxacin or trimethoprim–sulfamethoxazole is recommended in pediatric patients with bacteremia, extra-intestinal infections, or immunocompromised hosts (Table 15.1). Production of beta-lactamases generally makes *Yersinia* resistant to penicillin and cephalosporins [144].

Escherichia coli

E. coli is an abundant facultative anaerobe and normal inhabitant of the human colon and typically colonizes the gastrointestinal tract within hours after birth [145]. It is a gram-negative, lactose-fermenting motile bacillus of the family *Enterobacteriaceae*. Currently, 186 somatic (O) and 53 flagellar (H) antigens are recognized [146]. *E. coli* includes a heterogeneous group of microorganisms capable of exerting various possible interactions with the host, ranging from a normal inhabitant to that of a highly pathogenic organism [147].

Six distinct categories of *E. coli* are currently recognized as diarrhea-associated pathogens: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAggEC), and enteroinvasive *E. coli* (EIEC) (Table 15.1). Worldwide, *E. coli* is one of the most frequent causes of bacterial gastroenteritis [148].

Enterotoxigenic *E. coli* (ETEC)

Among the six recognized diarrheagenic strains of *Escherichia coli*, ETEC has been shown to be the most common bacterial enteric pathogen in several developing countries [149]. Transmission of ETEC occurs by ingestion of contaminated food and water, most commonly in resource-limited settings. ETEC is also an important cause of traveler's diarrhea with the incidence of ETEC in travelers from industrialized regions to developing countries estimated to be between 30% and 45% [150]. ETEC requires a relatively high inoculum and has a short incubation period (10–72 hours). The key symptom is watery diarrhea, which may also be accompanied by fever, abdominal cramps, and vomiting. In its most severe form, ETEC can cause cholera-like severe diarrhea and dehydration. The illness is generally self-limited, lasting less than 5 days. It is not uncommon for children in the developing world to experience 7 or more severe episodes of diarrheal illnesses in a year, with several due to ETEC [151]. Thus, ETEC has been associated with short- and long-term nutritional consequences in infants and children.

The pathogenesis of disease due to ETEC consists of ingestion of bacteria, intestinal colonization via expression of fimbriae, and the manifestation of virulence factors. ETEC strains elaborate on two classes of enterotoxins, namely, the heat-labile (LT) and the heat-stable (ST) enterotoxins [152]. LT is similar to cholera toxin (CT) and induces chloride secretion and inhibits sodium absorption by increasing the intracellular cAMP levels. ST is a small peptide that causes an increase of intracellular cGMP to also induce secretion of free water into the intestinal lumen causing secretory diarrhea [153]. The jejunum is a major target of ST-induced anion secretion that is mediated by CFTR. ST binds to its receptor guanylate cyclase C (GCC) on the apical surface of enterocytes, resulting in the generation of cGMP. This in turn activates a cGMP-dependent kinase (cGKII) leading to an

intracellular signaling cascade that ultimately leads to chloride efflux from the CFTR and inhibition of sodium uptake through the apical membrane of jejunal enterocytes [154].

Enteropathogenic *E. coli* (EPEC)

EPEC was the first group of *E. coli* serotype shown to be pathogenic for humans and has been responsible for devastating outbreaks of nosocomial neonatal and infant diarrhea worldwide, occurring most commonly in children less than age 2 [155]. EPEC is a leading cause of persistent diarrhea (lasting 14 or more days) in children in developing countries and may be associated with the development of malnutrition [156]. EPEC has a short incubation period (9–12 hours) before the development of non-bloody watery diarrhea. Additional symptoms may include fever, abdominal cramps, and vomiting.

EPEC strains are defined by the characteristic "attaching and effacing" (A/E) effect they elicit and by their inability to produce Shiga toxin. The ability to produce A/E lesions, which is characterized by microvilli destruction, direct interaction with epithelial cells, and effacement of microvilli, has also been detected in strains of Shiga toxin-producing *E. coli* (enterohemorrhagic *E. coli* [EHEC]) [157, 158]. Two subclasses of EPEC have been distinguished based on the presence of certain virulence factors: typical EPEC (tEPEC) and atypical EPEC (aEPEC). Typical EPEC strains possess a large virulence plasmid called EPEC adherence factor plasmid (pEAF) that encodes bundle-forming pili (BFP), while the atypical EPEC lack the pEAF. The locus of enterocyte effacement (LEE) is a specific chromosomal region containing genes necessary for formation of the A/E lesion as well as the T3SS [159]. The T3SS mediates the translocation of effector proteins from the bacterial cytosol into the infected cells.

A variety of mechanisms contribute to disease establishment and EPEC-mediated diarrhea illness. Microvilli destruction is responsible for the loss of absorptive surface and osmotic diarrhea. Intestinal pathogens can also disrupt the barrier by directly altering the distribution or phosphorylation of TJ proteins [160]. EPEC is also able to directly modulate host cell electrolyte transport and water transport, thereby contributing to the establishment of diarrhea [161]. These observations suggest that EPEC-induced diarrhea is a multifactorial process with alterations of electrolyte, solute, and water transport.

Diagnostic techniques such as single-target, multiplex, and quantitative PCR assays have become more widely available for the identification of EPEC [162]. Typical EPEC can be identified by a molecular detection panel specific for the pEAF. In contrast, tests that target the LEE (via detection of the *eae* gene, which encodes intimin) can identify isolates that lack the pEAF (by definition, aEPEC). Notably, the cause–effect relationship between EPEC detection in the stool and diarrhea is hampered by the high number of healthy carriers [163].

Enterohemorrhagic *E. coli* (EHEC)

EHEC is another major intestinal pathogen that is a subset of Shiga toxin-producing *E. coli* (STEC). *E. coli* O157:H7 is a particularly virulent EHEC serotype, although non-O157 serotypes also account for a large number of infections [164].

EHEC adheres to epithelial cells, expresses a T3SS, and causes "attaching and effacing" lesions much like EPEC. Unlike EPEC, infection with EHEC may cause severe symptoms including bloody diarrhea and life-threatening hemolytic uremic syndrome (HUS). These symptoms are due to the production of Shiga toxin which elicits luminal fluid accumulation in the intestine [165, 166]. The predominant transmission is through the ingestion of contaminated food. Crampy abdominal pain and non-bloody diarrhea are the first symptoms, sometimes associated with vomiting. Diarrhea always becomes bloody and abdominal pain worsens, lasting 1–22 days. Fever is usually absent or low grade [167].

In children, HUS is the most dangerous complication and is related to the circulation of Shiga toxins. Toxin-mediated microangiopathic injury leads to a prothrombotic state in the host and the formation of microthrombi. If extensive, these intravascular microthrombi can cause acute kidney injury [168]. HUS is usually diagnosed between 5 and 12 days after the onset of diarrhea. Risk factors include young age, bloody diarrhea, fever, an elevated leukocyte count, and treatment with antibiotics or antimotility agents [166, 169]. If a child has a recent diarrheal illness and presents with oliguria, paleness, easy bruising, or lethargy, evaluation for HUS is warranted. The classic trial of HUS includes hemolytic anemia, thrombocytopenia, and elevated creatinine [170].

Diagnosis of EHEC is made by stool culture or rapid stool assay for Shiga toxin. Reliable diagnostic testing early in illness is paramount and is associated with attenuated renal injury in *E. coli* O157:H7 infections [171].

Diffusely Adherent *E. coli* (DAEC)

DAEC comprises strains defined by a pattern of expressing Afa/Dr adhesins (Afa/Dr DAEC), in which the bacteria adhere to the epithelial cells in a diffused distribution [172]. Although the implication of DAEC strains in diarrhea remains controversial, multiple studies have confirmed the relationship between Afa/Dr DAEC and

diarrhea in children. In particular, the diarrhea illness susceptibility is age related and has been demonstrated to show an increased incidence in children <1 to 5 years of age [173]. The gastrointestinal symptoms include self-limiting watery diarrhea rarely associated with vomiting or abdominal pain. The diagnosis is mainly based on the DNA probe technique and on the pattern of adherence of the microorganism to HEp-2 cells [174, 175]. Given the technical problems of both assays, their use is limited to epidemiological surveys.

Enteroaggregative *E. coli* (EAEC)

EAEC are diarrheagenic bacteria defined by the characteristic aggregative adherence (AA) pattern on HEp-2 epithelial cells in culture. They have been especially associated with cases of persistent diarrhea in both the developed and developing world [176–178]. EAEC is transmitted by the fecal–oral route by food or contaminated water. Typical clinical features are a watery, mucoid, secretory diarrheal illness with low-grade fever, and little or no vomiting. However, bloody stools have been reported [179].

EAEC is detected by the classic cultured AA pattern in human epithelial cells or on a glass substrate in a distinctive stacked-brick formation. However, this gold standard method is time-consuming and DNA probes or a multiplex PCR is recommended to increase the ability to detect EAEC strains [180].

Enteroinvasive *E. coli* (EIEC)

EIEC strains are genetically, biochemically, and clinically nearly identical to *Shigella* [181]. They also show a similar epidemiological pattern and are endemic in developing countries. The role of EIEC in industrialized countries is limited to rare foodborne outbreaks. EIEC can rarely produce dysentery [182].

Treatment of Diarrheagenic *E. coli*

Generally, antibiotics should not be routinely administered for the treatment of *E. coli*. Although antibiotics may be effective in reducing diarrhea duration, most causes of *E.coli*-associated diarrhea will self-resolve [183]. In addition, antibiotic therapy is associated with antibiotic resistance and other adverse effects. For example, antibiotic therapy for Shiga toxin-producing *E. coli* (EHEC 0157:H7) does not significantly affect the clinical course or duration of fecal excretion of the pathogen and can increase the risk of HUS [184].

Antibiotic therapy is reasonable in patients with non-STEC *E coli*-associated diarrhea if symptoms are severe (i.e., associated with fever, requiring hospitalization) or diarrhea is bloody [59]. A lower antibiotic threshold is reasonable in immunocompromised patients [185]. Azithromycin is the treatment of choice for children. Fluoroquinolones had long been the first choice for treatment of travelers' diarrhea, but there has been emergence of resistance to this antibiotic class. TMP-SMX and ampicillin have also developed resistance worldwide, especially in children [186, 187].

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Intestinal Parasites

16

Phoebe Hodges and Paul Kelly

Introduction

Diarrhoeal diseases are the second leading cause of mortality in children under 5 years globally and kill approximately 525,000 children under the age of 5 annually [1]. A significant proportion of diarrhoeal disease in children is caused by parasitic infections, principally amoebae, flagellates and coccidia. Helminth infections, when heavy, can cause specific clinical syndromes. Parasitic infections can contribute to impaired growth and development in children which in turn has implications for cognitive function, education and economic productivity in later life [2].

Protozoa

Amoebae

Several species of *Entamoeba* are known to infect humans (see Table 16.1) although some of these species are likely to be non-pathogenic. It was previously thought that only a small proportion of those infected with *Entamoeba histolytica* showed symptoms, however, it later became apparent that many asymptomatic individuals were in fact colonised by a noninvasive parasite *E. dispar*, the cysts of which are morphologically indistinguishable from those of *E. histolytica* [3]. These cysts are typically between 10 and 15 μm in size and contain up to four 'ring and dot' nuclei. Other species with morphologically identical cysts were later described (*E. moshkovskii* and *E. bangladeshi*), and this has led to a change in the nomenclature when reporting this finding on stool microscopy to *E. histolytica/E. dispar/E. moshkovskii/E. bangladeshi* or E. complex [4]. These other species are considered non-pathogenic or less pathogenic; however, more

Table 16.1 Entamoeba and its characteristic

Entamoeba species	Characteristics and disease
E. histolytica	Causes invasive amoebiasis (haemorrhagic colitis or hepatic amoebiasis) in about 10% of carriers, many of whom have mild symptoms or are asymptomatic
E. dispar	Confirmed to be a separate entity from *E. histolytica* in 1993 [3], appears to be non-pathogenic, may be as many as 450 million new infections each year
E. moshkovskii	Reported to be present in 21% of stool samples from pre-school age children in Bangladesh [7] and in 15.9% of stool samples from patients attending rural clinics in northern South Africa [8] but was not significantly associated with diarrhoea, although in other studies an association with diarrhoea has been found [9, 10]
E. Bangladeshi	Initially identified in 2012 in stool samples from children living in Mirpur, Bangladesh [11], this species has been isolated from stools of not only asymptomatic children but also from those with diarrhoea
E. coli	Cysts are 15–30 μm in diameter with up to eight ring and dot nuclei
E. hartmanni	Smaller cysts (<10 μm) with up to four ring and dot nuclei. Generally considered to be non-pathogenic although a correlation was found between diarrhoea and *E. hartmanni* colonisation in school children in Indonesia [12]
E. gingivalis	Identified in periodontal pockets in patients with periodontitis [13]
E. polecki (subtypes 1–4)	Non-pathogenic species producing uninucleate cysts. Four subtypes have been described, of which subtype 4 appears to be the commonest in humans [14]
E. chattoni	Now referred to as *E. polecki* subtype 2
E. Struthialis	Now referred to as *E. polecki* subtype 3

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evidence on this subject is required. Of those colonised with *E. histolytica,* many will not experience symptoms and only about 10% will develop invasive amoebiasis (haemorrhagic colitis or hepatic amoebiasis). This is partly dependent on individual host factors and immune responses, for example, invasive amoebiasis occurs more frequently in men for reasons that are not yet understood. People who develop amoebic liver abscess have been found to have significantly higher titres of IgG1, IgG2 and IgG3 than individuals who are asymptomatic carriers of *E. histolytica* and interleukin (IL-4) levels are higher in patients with invasive amoebiasis [5]. Higher levels of interferon-gamma production, which are associated with nutritional status, have also been associated with reduced susceptibility to amoebic dysentery [6].

In some settings, *Entamoeba histolytica* is one of the commonest pathogens isolated in children presenting with diarrhoea. A study of aetiology of diarrhoea in Bangladeshi infants identified *E. histolytica* as the second most common pathogen after rotavirus in moderate-to-severe episodes of diarrhoea [15]. In primary school children in South West Ethiopia, it was found to be the commonest intestinal parasitic infection excluding helminthic infections [16]. It is recognised that malnourished children are particularly susceptible to amoebiasis and this may be due to heterogeneity in signalling pathways associated with leptin, a nutritional hormone which is suppressed in malnutrition. It was demonstrated in a cohort of Bangladeshi children that a polymorphism in the leptin receptor was associated with increased susceptibility to amoebiasis [17].

Amoebiasis is transmitted by ingestion of cysts in food or water contaminated with human faeces hence although its distribution is worldwide, it is more commonly seen in geographical areas where sanitation is poor. In travellers returning from low-income countries to high-income countries who present with gastrointestinal symptoms as a result of infection, *E. histolytica* has been found to be the third most commonly isolated pathogen [18]. Amoebiasis affects as many as 50 million people globally each year and has been estimated to cause 55,000 deaths annually [19, 20].

Following ingestions of cysts, excystation occurs in the terminal ileum or colon to give rise to the motile trophozoite stage which adhere to colonic epithelial cells through interaction of the galactose and *N*-acetyl-D-galactosamine-specific lectin (Gal/GalNAC lectin) and lipopeptidophosphoglycan on the surface of the trophozoite and toll-like receptor (TLR) 2 on intestinal epithelial cells [21]. KERP1 is another protein of the trophozoite membrane which binds to the brush border, and has been identified as a potential virulence factor in *E. histolytica* as expression of the gene *kerp1* has been linked to amoebic liver abscess in an animal model [22]. Cysteine proteases and in particular CP-A5 have also been identified as important virulence factors of *E. histolytica* involved in destruction of the extracellular membrane and tissue invasion through proteolytic activity. Located at the amoebic surface, CP-A5 binds to enterocytes triggering a pro-inflammatory response via phosphatidylinositol 3-kinases (PI3) kinase/AKT signalling [23].

Invasion of trophozoites through the mucosa and into the submucosal tissues gives rise to the pathognomonic flask-shaped ulcers. Presenting symptoms are classically bloody diarrhoea, abdominal pain and weight loss. A small percentage may develop complications such as fulminant amoebic colitis, toxic megacolon or amoebomas (granulation tissue within the wall of the colon which can lead to obstruction). Trophozoites penetrating into the portal system and thereby into the liver cause hepatocyte necrosis and abscess formation, which is uncommon in children and more frequently seen in adult males. Patients who develop amoebic liver abscess may do so many years after living in or travelling to endemic areas, and at this stage, stool microscopy is likely to be negative for cysts or trophozoites (Fig. 16.1).

Diagnosis of *E. histolytica* infection using stool microscopy alone is problematic for the reasons outlined above, namely, that cysts of *E. histolytica* are morphologically indistinguishable from cysts of at least three other species. Identification of trophozoites is diagnostic, however, these are usually only seen in stool samples examined within 30 minutes. Antigen detection enzyme-linked immunosorbent assay (ELISA) tests can be used; however, these are less sensitive than real time polymerase chain reaction (PCR) which can distinguish between *Entamboeba* species and is the gold standard for diagnosis. Serology should be carried out in patients with suspected amoebic liver abscess as it is almost always positive in these cases. In patients with acute colitis, flexible sigmoidoscopy and biopsy may be indicated to distinguish, for example, between other conditions such as inflammatory bowel disease [24].

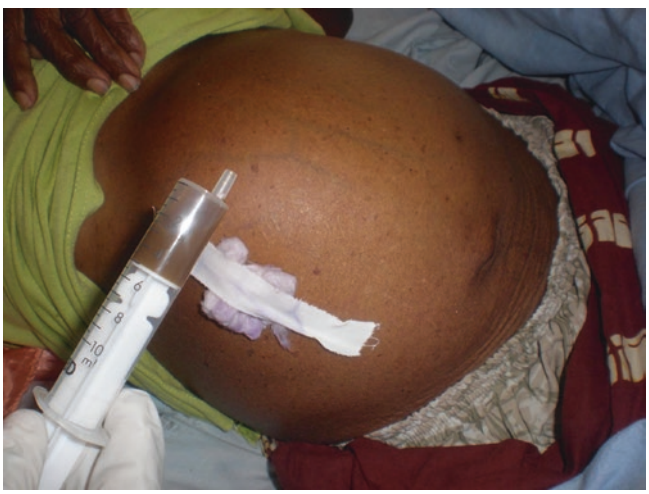

Fig. 16.1 Aspirate from an amoebic liver abscess, often described as resembling 'anchovy sauce'

Treatment is with metronidazole or tinidazole followed by a luminal amoebicide such as diloxanide, iodoquinol or paromomycin. Asymptomatic carriers of *E. histolytica* should be treated with a luminal amoebicide alone to prevent onward transmission.

Endolimax nana

Endolimax nana is an intestinal protozoon, the cysts of which are distinct as they are smaller than those of other intestinal amoebae (7–10 micrometres) and contain four nuclei. As with *Entamoeba*, it is spread via the faecal-oral route through ingestion of contaminated food or water. Studies of school-aged children in Côte d'Ivoire have found the prevalence of this intestinal parasite to be greater than 80% [25, 26]. Global prevalence has been estimated to be around 3.4% [27]. *Endolimax nana* is generally considered to be a non-pathogenic parasite although there are case reports and series linking co-infection with *Endolimax nana* and *Blastocystis* with diarrhoeal symptoms [28, 29].

Iodamoeba bütschlii

Iodamoeba bütschlii, another non-pathogenic intestinal amoeba, can be identified by its oval cysts 9–12 micrometres in size containing a glycogen mass, often referred to as a vacuole, which stains brown with iodine. The trophozoite stage is found in the colon while the cyst stage is responsible for transmission [30]. *Iodamoeba bütschlii* was identified in stool samples of 11.9% of school-aged children in a study of intestinal parasite infections in a highland district of Peru, however, prevalence of diarrhoeal symptoms was not reported in this study [31].

Giardiasis

Giardia intestinalis (Fig. 16.2) (also known as *G. lamblia* or *G. duodenalis*) is a flagellate protozoon which is transmitted by ingestion of infective cysts in contaminated food or water or from hands or fomites. Ingestion of as few as ten cysts constitutes an infective dose. Cysts are oval and measure between 8 and 12 μm with immature cysts containing two nuclei and mature cysts containing four 'ring and dot' nuclei. Excystation occurs in the small intestine, releasing motile trophozoites which are pear-shaped, measure 12–15 μm in size with eight flagella and contain two nuclei. These divide by longitudinal binary fission and can attach to the mucosa of the proximal small bowel via a ventral adhesive disk. Colonisation of the small bowel by this organism results in malabsorption and some of the symptoms of giardiasis include diarrhoea, foul-smelling stools, abdominal bloating and cramping, flatulence, malaise and weight loss.

Given that giardiasis is spread by the faecal-oral route, as with amoebiasis, prevalence would be expected to be highest in countries with poorer sanitation and hygiene standards. A review of outbreaks of water-borne parasitic infections between 2004 and 2010 found that the majority of reported outbreaks in fact occurred in high-income nations. Of the 22 reported outbreaks of giardiasis, only two were from outside the US, Europe or Australasia, however, this is extremely likely to be due to greater access to facilities for detection, surveillance and reporting in these settings [32]. The majority of these outbreaks were due to contaminated or inadequately treated drinking water sources; however, one outbreak in Trondheim, Norway originated from a child day care centre. Prevalence of giardiasis among children in limited-resource settings is likely to be extremely high, at least when measured by molecular diagnostics. The Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) projects, which were conducted at various sites in India, Bangladesh, Brazil, Nepal, Tanzania, South Africa, Pakistan and Peru, found that giardia was detected at some stage in the stool samples of two-thirds of the 1741 children who were followed up to 2 years of age [33] (Fig. 16.2).

Diagnosis is by identification of cysts on wet preparation microscopy, although sensitivity may be hindered by the intermittent nature of cyst shedding from the gut, and at least three stool samples taken on separate days should be examined (as for optimum diagnosis of most parasites). The sensitivity of microscopy in the MAL-ED study was found to be 46% when compared to enzyme immunoassay. Other more costly methods of diagnosis with higher sensitivity include direct fluorescence antibody staining of cysts, antigen detection by enzyme-linked immunosorbent assay (ELISA), small bowel biopsy or PCR.

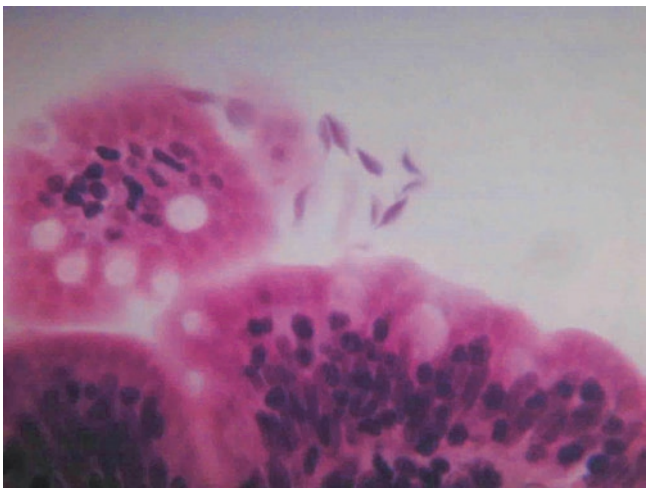

Fig. 16.2 Small bowel biopsy with trophozoites of *Giardia intestinalis* seen in cross section

As with *Entamoeba histolytica*, the clinical course and symptomatology in those colonised with *G. intestinalis* differs from individual to individual. Studies of localised outbreaks show that some individuals develop an acute self-limiting diarrhoeal illness, some develop persistent diarrhoea with malabsorption and others are asymptomatic [34, 35]. Whilst giardiasis is certainly a well-recognised cause of diarrhoea in travellers returning from endemic areas [36], there is much debate about whether paediatric giardiasis in high-prevalence areas is associated with acute diarrhoeal illness. In MAL-ED, detection of *G. intestinalis* in surveillance stool samples was not found to be associated with an increased risk of acute diarrhoeal illness. It was, however, found to be associated with poorer growth outcomes (weight and length attainment) at 2 years of age. Detection of *G. intestinalis* was also found to be associated with an increase in intestinal permeability as measured by the lactulose mannitol recovery ratio, a marker which is often used as a surrogate measure of environmental enteric dysfunction. This may partly account for the poorer growth outcomes in these children [33].

Cyst shedding in adults who are infected with *G. intestinalis* is at lower levels than in children, suggesting that some degree of immunity is acquired over time in those who are continually exposed to this pathogen. Individuals who are immunocompromised are more likely to suffer from severe or prolonged giardiasis due to reduced production of anti-*Giardia* antibodies [37]. In addition to antibody production, a role has been identified for mast cell production of IL-6 in the immune response to *Giardia* [38].

Treatment of giardiasis is with metronidazole or tinidazole, or single dose albendazole for a duration of 5–10 days which has similar efficacy. Increasing numbers of treatment-refractory cases of giardiasis are, however, being noted, with rates of cases refractory to treatment with nitroimidazoles registered at the Hospital for Tropical Diseases in London rising from 15% in 2008 to 45% in 2013 and the majority of these cases originating from Asia [39]. Following a waterborne outbreak of giardiasis in Bergen, Norway, a cohort of patients experiencing persistent gastrointestinal symptoms despite metronidazole were investigated and ongoing infection with *G. intestinalis* was found in 32.3% of patients [35]. However, there are also high rates of functional gastrointestinal disease (FGID) and post-infectious chronic fatigue syndrome in people who have previously been infected but have cleared the infection. In people who had laboratory-confirmed giardiasis during the 2007 outbreak in Bergen, immunological differences were found between those who went on to develop FGID symptoms and those who did not, with significantly increased levels of CD8 T cells found in those with FGID, 5 years after the outbreak [40].

Dientamoeba fragilis

This is a flagellate protozoon related to trichomonads, which takes its name from its rapid degeneration or 'fragility' when outside the human body. It has only recently become recognised as a probable pathogen as opposed to a commensal of the human gastrointestinal tract, with correlations made between *D. fragilis* carriage and symptoms of abdominal pain and diarrhoea and resolution of symptoms coinciding with *D. fragilis* clearance following antibiotic treatment. Trophozoites are binucleate measuring 9–12 μm and can be identified by light microscopy after fixation and staining, however, *D. fragilis* can also be detected using PCR techniques. The mechanism of transmission of *D. fragilis* in humans is incompletely understood. Although a cyst stage has been described in stool samples from patients known to be infected, this was at a prevalence of only 0.01%, making the role of the cyst stage in transmission uncertain. The prevalence of pseudocysts or precysts was much higher at 32.6% [41]. One theory relating to the transmission of *D. fragilis* is that the organism may be transmitted via a vector, with *Enterobius vermicularis* eggs having been proposed for this role and the detection of *D. fragilis* DNA in *Enterobius* eggs supporting this hypothesis [42].

There is a growing body of evidence for the pathogenicity of *D. fragilis*. A study of 695 children in California conducted from 1976 to 1978 found *D. fragilis* in 9.4% based on examination of stool samples, and 91% of these children were found to be suffering from gastrointestinal symptoms including abdominal pain, diarrhoea and anorexia. Half of these children were also noted to have a peripheral eosinophilia. Symptoms were found to be lessened or resolved following treatment with metronidazole or diiodohydroxyquin [43]. In a more recent study of school children in Sweden, prevalence of *D. fragilis* among children in two schools was found to be 60%, significantly higher than the 15% prevalence found in clinical samples submitted to the regional laboratory for parasitological examination. Prevalence was higher (95%) amongst children who were found to have *Enterobius vermicularis*, supporting the hypothesised role for this helminth in transmission of *D. fragilis*, and also in children who complained of gastrointestinal symptoms, supporting the pathogenicity of this organism [44].

It seems reasonable that in patients with gastrointestinal symptoms in whom all other causes have been ruled out, treatment of dientamoebiasis should be prescribed. Various antimicrobial agents have been reported as successfully leading to elimination of *Dientamoeba* infection, including clioquinol, iodoquinol, metronidazole, paromomycin, secnidazole and ornidazole, however, there is little evidence as regards the relative efficacy of these treatments [45].

Balantidium coli

This is the only ciliate known to infect humans and is the largest of the gastrointestinal protozoa, with the ciliated motile trophozoite measuring 60–70 μm and the cyst measuring 50–60 μm in diameter. Both cysts and trophozoites are readily recognisable by wet preparation microscopy.

The reservoir host of this organism is the pig and infections are commoner in individuals who have contact with these animals, although human-to-human transmission can occur. Distribution of *B. coli* is worldwide and prevalence has been estimated at between 0.02% and 0.1%. *B. coli* seems to have a low level of virulence, and infection in humans may be subclinical although dysentery and death can occur, with mortality resulting from haemorrhagic dysentery and shock or colonic perforation. Treatment with metronidazole is effective [46].

As with other gastrointestinal protozoan infections, outbreaks are more frequent in developing countries where people are more likely to live in close proximity with their livestock and the water supply may be contaminated with human or porcine faecal matter. A large outbreak of balantidiasis involving 110 people on the island of Truk in the Western Pacific, where many residents kept pigs, occurred after a typhoon hit the island resulting in the destruction of usual water collection systems so that residents were forced to use contaminated water from streams and wells [47]. However, *B. coli* has also been described as a commensal in such populations. A study of 2124 apparently healthy children from rural Aymara Indian communities in Bolivia where the main economic activities are livestock breeding and agriculture revealed an overall prevalence of *B. coli* of 1.2% with more than half of the pigs in these communities being found to be infected. The authors concluded that *B. coli* should be considered an endemic anthropozoonosis in this region, and it is likely to have similar endemicity in other regions where the environment is highly contaminated with faecal material. However, it should be noted that 30% of the children in this study had malnutrition and were heavily parasitised with protozoan and helminth species, shedding doubt on the apparent commensalism of this organism [48].

Blastocystis

Previously known as *Blastocystis hominis*, but now undergoing taxonomic revision through molecular analysis, this is a genetically diverse stramenopile with at least 17 different subtypes which are microscopically indistinguishable. The pathogenicity of this organism remains unclear but it is very prevalent amongst humans, with one study reporting universal prevalence in children living in the Senegal River Basin [49]. As with other intestinal parasites, prevalence in developed countries has been shown to be significantly lower, with prevalence in Europe, for example, being estimated at 30% [50]. Diagnosis is by microscopy of wet-film preparations or fixed and stained preparations, however, this method has been found to have a sensitivity of only 48% as opposed to 98% diagnostic sensitivity with PCR [51]. *Blastocystis* has been implicated in gastrointestinal disease, including irritable bowel syndrome (IBS) and a putative condition which has been termed 'blastocystosis', symptoms of which have been described to include abdominal pain, bloating, diarrhoea and flatulence [52], however, there is no definitive evidence for its role as a pathogenic agent.

First-line treatment is with metronidazole but the efficacy of this is variable and persistence of symptoms following treatment has been reported suggesting resistance or incomplete clearance of the organism, with one study even demonstrating increased rates of growth of *Blastocystis* following treatment with low concentration metronidazole [53]. Trimethoprim–sulfamethoxazole and paromomycin have also been used to some effect [54]. Antimicrobial treatment should only be prescribed in symptomatic individuals in whom no other potential causative organisms have been identified.

Coccidia

These are intracellular parasites which can infect immunocompetent individuals but are of greater clinical concern in the immunocompromised, in whom they can cause severe and prolonged infections. Transmission is by the faecal oral route with sporozoite-containing, resistant oocysts being shed in the faeces and ingested in faecally contaminated water or food. Following ingestion, excystation occurs and sporozoites are released which then adhere to enterocytes and follow either a perpetuating asexual reproductive cycle or a sexual reproductive cycle giving rise to the formation of sporozoite-containing oocysts.

Cryptosporidium (Fig. 16.3)

Cryptosporidium parvum and *C. hominis* are responsible for the majority of cases of cryptosporidiosis in humans, however, there are a number of other species which can also infect humans. The Global Enteric Multicenter Study (GEMS) which investigated the aetiology of diarrhoeal disease in children in sub-Saharan Africa and South Asia found that *Cryptosporidium* was the second most common causative organism in infants of 0–11 months [55]. In developed countries where cryptosporidi-

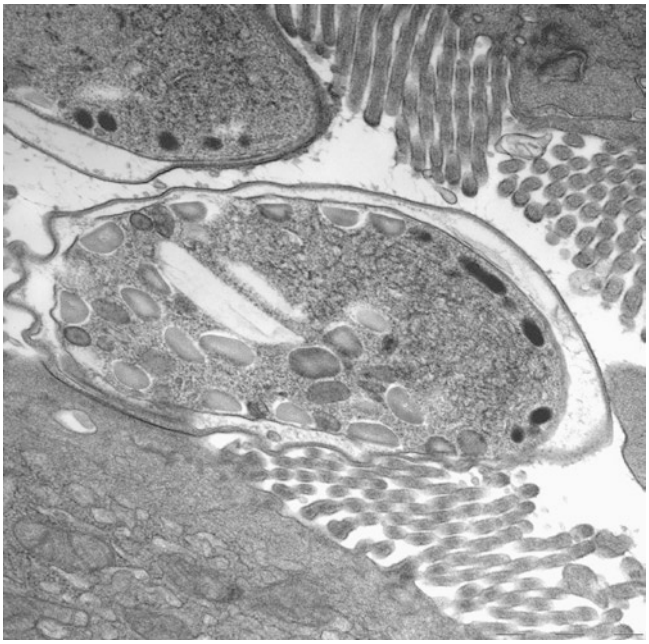

Fig. 16.3 Transmission electron micrograph showing cryptosporidium on intestinal epithelial cell

osis is not endemic, localised outbreaks may occur centred around childcare settings or swimming pools, while larger water-borne outbreaks related to contamination of public water supplies have been reported in the US and Europe [56, 57]. Cryptosporidiosis causes watery diarrhoea and is self-limiting in immunocompetent individuals with symptoms lasting up to 2 weeks, but can have a more severe course in those who are immunocompromised, spreading to extraintestinal sites including the biliary tract and rarely the lungs [58].

Diagnosis can be made by microscopic identification of oocysts 5–6 ⌠m in diameter in the stool after modified Ziehl-Neelsen or auramine staining. Direct fluorescence antibody tests for faecal antigens are also available, and PCR is widely used for diagnosis as well as genotyping.

Nitazoxanide can reduce the duration of symptoms and infectious period for immunocompetent individuals, however, it has not been found to have any benefit in children who are human immunodeficiency virus (HIV) seropositive even at high doses with prolonged treatment of up to 28 days [59, 60]. In this setting, the mainstay of treatment would be initiation of antiretroviral therapy. Immune reconstitution to a CD4 count of >100 cells/mm^3 usually results in resolution of symptoms.

Cyclospora cayetanensis

This is a small bowel parasite, the oocysts of which are distinguishable from those of cryptosporidium by their slightly larger size (8–10⌠ m). Oocysts of *Cyclospora* stain variably with modified Ziehl-Neelsen and safranin reportedly produces a more consistent stain [61]. Cyclosporiasis presents with symptoms of abdominal cramps, anorexia, nausea, weight loss and prolonged diarrhoea, which can last for up to 6 weeks although duration of symptoms is reportedly longer in those who are immunosuppressed [62]. In areas of endemicity, children younger than 5 years seem to be at higher risk of experiencing symptoms of diarrhoea in relation to *Cyclospora* infection, and it may be the case that recurrent exposure to this pathogen at an early age results in a degree of immunity and asymptomatic infection in later life [63]. In non-endemic areas, *Cyclospora* has been identified as a cause of diarrhoea in travellers returning from endemic area, with seasonal outbreaks reported consistently in recent years in travellers returning from certain areas of Mexico suggesting that these outbreaks may be related to a contaminated food or drink item being supplied to hotels in these regions [64]. Outbreaks have also been reported as a result of the importation of salad and soft fruit from endemic regions [65]. Treatment is with trimethoprim–sulfamethoxazole which should be continued as secondary prevention in immunosuppressed individuals.

Cystoisospora belli

Oval oocysts measuring 25–30⌠m can be detected in the faeces by microscopy following staining with iodine or Ziehl-Neelsen staining. Sporozoite-containing sporocysts may be visible within these oocysts. Commercial PCR assays are also available. As with *Cryptosporidium*, this parasite is endemic to tropical and subtropical areas and causes self-limiting watery diarrhoea in immunocompetent individuals with symptoms lasting for up to 10 days. It is an uncommon cause of diarrhoea in immunocompetent children even in endemic regions (isolated in 2% of stool samples of children presenting with diarrhoea to a hospital in New Delhi, India [66] and is of greater clinical significance in HIV-positive adults in low-income settings [67]. Treatment is with trimethoprim–sulfamethoxazole which can be continued as secondary prevention in patients who are immunocompromised.

Helminths

Nematodes (Roundworms)

Enterobius vermicularis (Pinworm) (Fig. 16.4)

Of all the helminths, this is the most widely distributed worldwide with around 300 million cases annually and is common in temperate as well as tropical regions. This parasite is particularly prevalent in children and occurs in outbreaks centred around group settings such as day care centres or playgroups. European frequency analyses place the prevalence of this parasite in school-age children somewhere between 12.7% and 28.5% [68, 69]. When eggs are ingested, they hatch in the small bowel and travel to the colon where

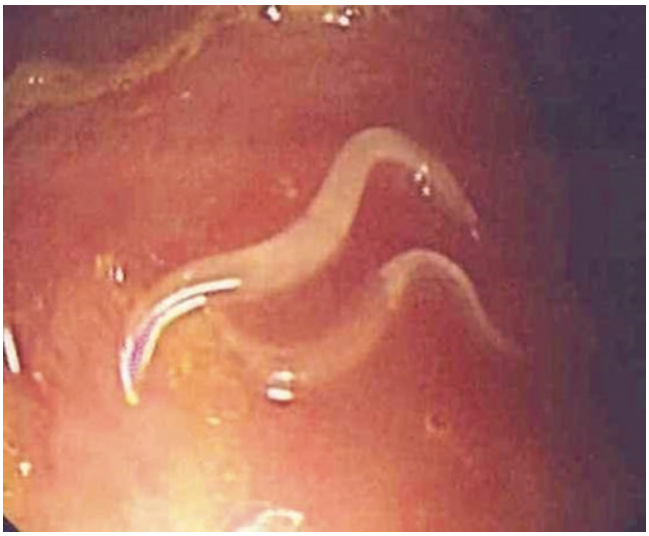

Fig. 16.4 Endoscopic image showing *Enterobius vermicularis* in the colon

adult worms live in the caecum and appendix. Gravid female worms migrate to the anal canal and lay their eggs in the perianal area. These eggs adhere to the skin by a glue-like matrix which promotes itching of the perianal area and transmission is often via contaminated hands. The most frequently reported symptom is pruritus ani which often leads to disturbed sleep as female worms predominantly lay their eggs at night. Extraintestinal manifestations are rare but reported, including vulvovaginitis and urinary tract infections from migration of worms with or without adherent bacteria into the vagina. Diagnosis is via identification of the eggs under a microscope. These can be collected by applying adhesive tape to the perianal region (Sellotape method) and then transferring onto a slide. Treatment is with a single dose of albendazole or mebendazole and good attention to hygiene including frequent washing of clothes and bed linen. The entire household should receive treatment and this should be repeated after 2 weeks due to the high rate of reinfection from residual eggs on fomites.

Ascaris lumbricoides

This is a soil-transmitted helminth, the eggs of which need contact with the soil in the optimal conditions of warm temperatures and moisture in order to mature and become infective. The soil-transmitted helminths largely affect populations of developing countries with tropical or subtropical climates, particularly the rural poor. The WHO estimates that 24% of the world's population are infected with soil-transmitted helminths, with *Ascaris* being the most prevalent of these. In regions where prevalence of any soil-transmitted helminth exceeds 20%, WHO recommends preventive chemotherapy for all preschool- and school-aged children with single dose albendazole or mebendazole on an annual basis or a biannual basis where prevalence exceeds 50% [70]. The evidence for benefit of mass drug administration for helminthic infections is mixed, with the DEVTA trial undertaken in north India being the largest trial to date to examine the effects of deworming on mortality in children. This cluster-randomised trial included one million children aged 1–6 years and found that 6 monthly treatment with albendazole reduced prevalence of worm infection by half but was not associated with any significant survival or growth benefit, although the authors noted that this was a lightly infected population [71].

Ascaris is transmitted via the faecal oral route when eggs are ingested. They hatch in the duodenum and larvae subsequently migrate through the gut mucosa into the portal circulation and then via the systemic circulation to the lungs, where they penetrate the alveoli, ascend the trachea and are swallowed. Migration of the larvae through the lung can result in pulmonary eosinophilia (Loeffler's syndrome) and symptoms of dry cough, shortness of breath and fever, however, the vast majority of infections are asymptomatic. Malabsorption in individuals with very heavy intestinal infection can contribute to malnutrition. Female worms can grow up to 40 cm in length and lay up to 200,000 eggs per day and if the worm burden is large, they can rarely cause obstruction of the small intestine leading to perforation. Other less common presentations resulting from migration of worms to extraintestinal sites include pancreatitis and biliary obstruction. Diagnosis is by identification of the characteristic ova in stool samples either by direct smear, Kato-Katz preparation or formol ether concentration. Treatment is with benzimidazole antihelminthic drugs; either a single dose of albendazole or mebendazole twice daily for 3 days.

Trichuris trichiura (Whipworm)

Also a soil-transmitted helminth, as with *Ascaris*, eggs of *Trichuris* become infective after contact with soil and when ingested, hatch in the small intestine. Adult worms are 3–5 cm long and burrow into the epithelium of the caecum and colon with the posterior end of the worm in the lumen, causing *Trichuris* colitis which may result in diarrhoea or dysentery, abdominal pain, anaemia, and in severe cases, rectal prolapse caused by oedema. Female worms produce 3000–5000 eggs per day and diagnosis is made by identification of these characteristic ova in stool specimens using the same techniques as described above for *Ascaris*. Benzimidazole treatment is commonly used but should be for 3 days as single dose treatment is much less effective at achieving parasite clearance than in ascariasis [72].

Hookworm

Necator americanus and *Ancylostoma duodenale* both fall into this category, with *Necator* being the more common of the two. As with the other soil-transmitted helminths, both are widespread throughout the tropics. Transmission commonly

occurs when infective larvae in the soil penetrate the skin, usually the soles of the feet, although ingestion of the larvae of *A. duodenale* also leads to infection. Larvae in the skin penetrate the blood vessels and migrate through the lung, ascend the trachea and are swallowed. In the small intestine, the larvae mature into adult worms which attach to the mucosa causing iron-deficiency anaemia. *A. duodenale* tends to cause more blood loss than *N. americanus* and the degree of anaemia is proportional to worm burden. Some groups are particularly susceptible to anaemia including children who are co-infected with other parasites such as malaria and *Schistosoma.* These worms can live for up to 10 years in the intestinal tract, causing chronic anaemia which can lead to heart failure and stunting in children. Other presenting symptoms include epigastric pain, dry cough caused by migration of the larvae through the lungs and itching at the site of larval penetration of the skin. In the case of dog or cat hookworms (*Anycylostoma braziliense* and *Ancylostoma caninum*), a migrating rash known as cutaneous larva migrans can occur. This is caused by movement of the larvae through the superficial layers of the skin. Albendazole is more effective for treatment of hookworm than mebendazole and combination therapies with pyrantel pamoate, oxantel pamoate and levamisole achieve an even higher cure rate and may help to reduce the risk of developing resistance to benzimidazoles [73]. Campaigns for increased use of footwear in at-risk populations are an important part of public health measures to control this disease.

Strongyloides stercoralis (Fig. 16.5)

Strongyloidiasis occurs generally in tropical or subtropical regions but has been described in more temperate climates including North America and Europe. *Strongyloides stercoralis* is a soil-transmitted helminth which infects humans when free-living larvae in the soil penetrate the skin. These larvae then migrate via the heart to the lungs, ascend the trachea and are swallowed. In the small intestine, adult female worms 2 mm in length invade the mucosa and can reproduce asexually, producing first stage larvae which are either passed in the stool to continue the external life cycle or mature into infective larvae within the host, penetrating the gut mucosa and thereby continuing a cycle of autoinfection which can lead to chronic infection of the host without re-exposure. These persistent infections have been observed to last for more than 50 years in some cases, for example, ex-prisoners of war on the Thai-Burma railway [74]. Although prevalence of strongyloidiasis seems to increase with age, high rates of infection of children in at-risk populations have been reported, for example, 10% prevalence was found in children aged 2–12 years in a rural Amazonian community in Peru [75] and 21% prevalence was reported in school children in Western Angola [76]. Although many acute infections are asymptomatic, some will experience diarrhoea and abdominal pain or rarely cough or wheeze due to migration of larvae through the lungs. Symptoms of chronic infections include larva currens (the pruritic rash caused by migration of larvae through the skin during autoinfection) and non-specific gastrointestinal symptoms including intermittent diarrhoea and malabsorption. A rare but sometimes fatal complication of strongyloidiasis is hyperinfection or disseminated strongyloidiasis which can occur in the immunosuppressed, particularly those with HTLV-1 co-infection or taking systemic steroid treatment. Hyperinfection occurs when massive larval migration takes place from the gastrointestinal tract to the peritoneum and other extraintestinal sites, resulting in peritonitis and Gram-negative septicaemia [77].

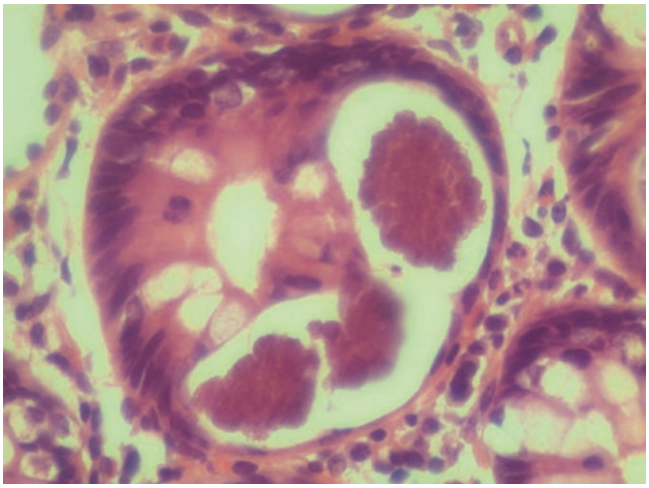

Fig. 16.5 Haematoxylin- and eosin-stained duodenal biopsy showing *Strongyloides stercoralis* adult worms

Diagnosis by stool microscopy is unreliable as larval shedding in the stool is intermittent, and other methods such as charcoal or agar plate culture, serological testing, or duodenal biopsy should be employed where available as adjuncts to direct stool microscopy. Ivermectin is the drug of choice for treatment of strongyloidiasis although thiabendazole appears to be equally effective in terms of cure rate but is associated with a higher rate of adverse events [78].

Cestodes (Tapeworms)

Taenia solium (pork tapeworm) and *Taenia saginata* (beef tapeworm) are transmitted to humans by ingestion of undercooked meat which contains cysticerci (larval stages) of the parasite. In the small intestine, the cysticercus develops into a tapeworm over a period of 10 weeks and attaches to the mucosa of the small intestine by means of suckers on the scolex (anterior end of the worm). These tapeworms can grow to considerable lengths, with adults generally being around 5 m in length, and consist of segments or proglottids

which mature and become gravid, eventually lysing and releasing eggs. Pigs and cattle become infected when they ingest vegetation contaminated with eggs or gravid proglottids which have been passed in faeces of infected humans. *Taenia solium* is of clinical significance as it can cause cysticercosis when cysts invade extraluminal tissues. Neurocysticercosis which occurs when cysticerci enter the brain is the foremost cause of acquired epilepsy in endemic regions, being responsible for about 30% all cases of epilepsy in studies from Latin America, sub-Saharan Africa and Southeast Asia [79]. Intestinal taeniasis does not usually cause symptoms, however, the host might observe tapeworm segments in the faeces, or occasionally experience weight loss or abdominal pain. Diagnosis can be made by identification of ova or gravid proglottids in faeces, and although the eggs of these two species are indistinguishable it is possible to distinguish between gravid proglottids of *T. solium* and *T. saginata* microscopically based on the number of uterine branches. Single oral dose praziquantel (10 mg/kg) is effective for treatment of intestinal infections, alternatively single oral dose niclosamide can also be used.

Diphyllobothrium latum (fish tapeworm) infects humans when they ingest undercooked fish containing plerocercoids (infective larvae of *D. latum*). Diphyllobothriasis does not generally result in symptoms although some may experience non-specific abdominal symptoms. As this parasite competes for host vitamin B12, infection can result in megaloblastic anaemia. Treatment is with single dose praziquantel or niclosamide as with taeniasis.

Hymenolepis nana (dwarf tapeworm) is a smaller species of tapeworm which grows up to 40 mm in length in the intestine and, although distribution is global, prevalence is particularly high in children living in areas with poor access to sanitation. For example, prevalence in children of one refugee population in Pakistan was shown to be 42% [80]. Humans become parasitised when they ingest arthropods containing cysticercoids which develop into adult tapeworms in the small intestine. Alternatively, infection can occur when an embryonated egg from contaminated food, water or fomites is ingested, or by autoinfection whereby eggs in the small intestine release first stage larvae which then penetrate the villus, develop into cysticercoid larvae and are released back into the intestinal lumen on rupture of the villus where they mature into adult worms and continue the infective cycle without passing through the external environment. Symptoms of headache, fever, fatigue and diarrhoea are reported. Treatment is with praziquantel 25 mg/kg as a single dose or alternatively with niclosamide or nitazoxanide.

Strategies for control and prevention of the parasites discussed above include inspection of meat, thorough cooking and/or freezing of fish and meat (freezing meat at -20C for 12 hours and fish at −18C for 24–48 hours), and improved hygiene and sanitation measures in populations.

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Persistent Diarrhea in Children in Developing Countries

17

Jai K. Das, Zahra Ali Padhani, and Zulfiqar A. Bhutta

Introduction

In the year 2018, 5.3 million children under the age of 5 years died [1]; around 29% of these deaths were due to diarrhea, pneumonia, and malaria [2]. After respiratory diseases, diarrhea constitutes a serious public health challenge, especially as it constitutes 63% of the global burden and a leading cause of death among children under the age of 5 years and is the second leading cause of infant mortality [3]. The diarrhea burden is specifically concentrated in Asia, Africa, and South America and is the cause of every one in eight child mortality annually of children under the age of 5 years [4, 5].

Diarrhea is defined as "the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual)" [6]. The common risk factors for diarrhea include young age, low socioeconomic status, suboptimal breastfeeding, early weaning, malnutrition, low maternal education, seasonal variations, lack of handwashing, poor hygiene and sanitation practices, and untreated water supply at home [3, 7–9].

Diarrhea has been classified into three types by common clinical criteria. *Acute watery diarrhea* is brief and lasts several hours or days (1–3 days) and is often due to gastrointestinal pathogens including viruses (rotaviruses and caliciviruses) and bacteria (including *Escherichia coli* and *Vibrio cholerae*). *Acute bloody diarrhea*, also called dysentery, is marked by stools containing blood and/or mucous; common pathogens include *Entamoeba histolytica* (amoebic dysentery) or *Shigella* species (bacillary dysentery). *Persistent diarrhea* refers to episodes of diarrhea (either watery or dysentery) that last 14 days or longer. It is majorly caused by *E. coli*, *Shigella* or *Cryptosporidium*. Although less common than acute diarrhea, prolonged and persistent episodes of diarrhea in childhood constitute a significant portion of the global burden of diarrhea and these lengthy episodes are increasingly implicated in childhood undernutrition [10], micronutrient deficiencies (such as folate, vitamin A, and zinc), immune deficiency, adverse neurodevelopment outcomes, and higher morbidity and mortality from other diseases [11]. As mortality from acute watery diarrhea is decreasing, the proportion of deaths due to persistent diarrhea has increased and recent studies estimate that between 5% and 18% of all episodes are persistent diarrhea, and though a small portion of episodes, they are responsible for significant diarrheal morbidity and up to 50% of all diarrhea-related deaths since persistent diarrhea has a high case fatality rate [12, 13]. It is estimated that diarrheal incidence and mortality among children under the age of 5 years are associated with 40.25 million disability adjusted life years (DALYs) lost in 2016 [14]. After accounting the long-term sequel of malnutrition and diarrhea, the DALYs double by 40% to 55.78 million years [14]. These findings indicate the continuing need to focus on prevention and management of childhood diarrhea, especially in developing countries where most of the burden lies. But there is scarcity of data on persistent diarrhea, and it is likely that the diminished publication output also reflects reduced research interest in the subject [15]. Although effective interventions exist, which can not only halt the progression from acute diarrhea to persistent diarrhea and its sequelae, but also have a substantial impact on total diarrhea burden and mortality, these interventions do not have universal access. The integrated Global Action Plan for the Prevention and Control of Pneumonia and Diarrhea (GAPPD) by the

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United Nations Children's Fund (UNICEF) reported that interventions such as handwashing with soap can reduce the risk of diarrhea by 31%, similarly other interventions like improved sanitation can reduce the risk by 36%, improved water quality can decrease the risk by 17%, oral rehydration solution (ORS) reduces the risk by 69%, zinc by 23%, and vitamin A by 23% [16]. There is a 10.5 times greater risk of mortality among children not breastfed during 0–6 months, with 28% (RR 2.28–10.52) risk of mortality due to diarrhea [16]. An analysis using the Lives Saved Tool (LiST), a methodology to estimate the effect of increasing the use of a package of interventions, has shown that if water, sanitation, and hygiene (WASH), breastfeeding, ORS, rotavirus vaccine, vitamin A supplementation, zinc for the treatment of diarrhea, and antibiotics for dysentery are scaled up to at least 80% and that for immunizations to at least 90%, then 95% of diarrheal deaths in children younger than 5 years could be eliminated [17].

Etiology

The cause of persistent diarrhea in developing countries is associated with serial enteric infection (without enough recovery time), which follows an acute episode of diarrhea [4]. In developed countries, children are not likely to be exposed to malnutrition and enteric infections, however, it is most likely to be caused due to celiac disease, food allergies, and inflammatory bowel disease (IBD). In developing countries, persistent diarrhea may increase the risk of malnutrition and intercurrent illnesses such as respiratory diseases [18].

Persistent diarrhea is usually caused by infections which includes bacteria (*Aeromonas*, *Campylobacter*, *C. difficile*, *E. coli*, *Plesiomonas*, *Salmonella*, *Shigella*), parasites (*Cryptosporidium, Cyclospora*, *E. histolytica*, *Giardia*, *Microsporidia*), and viruses (rotavirus, norovirus) [3]. A few pathogens have been particularly associated with persistent diarrhea or are preferentially identified when an episode becomes persistent, such pathogens include enteroaggregative *E. coli* (EAEC), and enteropathogenic *E. coli* (EPEC), *Cryptosporidium*, *Shigella*, *Campylobacter*, *Yersinia*, and *Giardia lamblia* [18–20]. These pathogens cause continuous damage to mucosal lining of the intestine.

The exact cause of persistent diarrhea within population is still poorly understood and individual mechanism of action is still unknown [19]. The original infectious agents are particularly virulent and difficult to clear (e.g., *Shigella* species) [18]. These agents cause destruction of villus tips which leads to intestinal damage and reduced intestinal absorptive surface area, which in turn interferes with the absorption of nutrients. Disrupted intestinal flora and delayed healing cause prolonged diarrhea and exposure to enteropathogens which cause a new infection before the recovery of the previous infection leading to persistent diarrhea [18].

Based on the pathogenic processes and clinical features, diarrheagenic *E. coli* (DEC) has been clustered into six pathotypes. The six pathotypes are EPEC, EAEC, enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), diffusely adherent *E. coli* (DAEC), and enteroinvasive *E. coli* (EIEC) [21–23]. In epidemic situations, *E. coli*, *Clostridium difficile*, and *Vibrio cholerae* have been reported as the causative agents of diarrhea in China [24, 25], Iran [26], Nigeria [27], and Yemen [28, 29]. In Bangladesh, *Cryptosporidium* (an intestinal parasite) was reported in chronic diarrheal cases, whereas *Giardia lamblia* was reported in acute and chronic cases of diarrhea in Peru [18, 30].

Risk Factors

The risk factors of persistent diarrhea includes medications (antibiotics), malnutrition, altered immune system, lack of access to clean water, poor sanitation, intolerance to food products (lactose, gluten), thyroid, metabolic and intestinal disorders, and reduced intestinal blood flow [3, 31, 32].

Persistent diarrhea is commonly seen in association with significant malnutrition and the relationship between persistent diarrhea and malnutrition is bidirectional. The evidence of micronutrient deficiencies, especially of zinc and vitamin A in malnourished children with persistent diarrhea, indicates impaired immunological mechanism that is associated with an increase in inflammatory mediators, leading to tissue damage caused by enteric infection. Malnutrition also increases the chances of death due to persistent diarrhea [3]. It impairs tissue repair mechanisms, and the infection tends to be more severe for longer duration. Lactose intolerance is prevalent in many children with persistent diarrhea, but the role of specific dietary allergies in inducing and perpetuating enteropathy of malnutrition is unclear. Several studies have highlighted the high risk of prolonged diarrhea with lactation failure and early introduction of artificial feeds in developing countries. In particular, the administration of unmodified cow or buffalo milk is associated with prolongation of diarrhea, suggesting the potential underlying role of cow's milk protein allergy (CMPA) and milk protein enteropathy [33]. Inappropriate management of acute diarrhea is also an important risk factor. The association of prolongation of diarrhea with starvation and inappropriately prolonged administration of parenteral fluids has been recognized for over half a century [34]. Continued breastfeeding is important, and unnecessary food withdrawal and replacement of luminal nutrients, especially breast milk, with non-nutritive agents is a major factor in prolonging the mucosal injury after diarrhea. In particular, routine administration of antibiotics, antimotility agents, and semistarvation diets should be

avoided in cases of prolonged diarrhea [35]. There is now clear evidence supporting the enteral route for nutritional rehabilitation of malnourished children with persistent diarrhea [36] as starvation has been shown to have deleterious effects on the intestinal mucosa [37] with a reduction in gastrointestinal structure and function. It is therefore imperative that malnourished children with persistent diarrhea should receive enteral nutrition during their period of rehabilitation. High stool frequency, not being breastfed, young age, and acquiring diarrhea in the rainy season have also been identified as risk factors for prolonged diarrhea [38].

HIV has also shown association with persistent diarrhea. It affects several cellular mechanisms, for example, it causes the release of transactivating factor protein (Tat) by the virus which impacts enterocytes, both as an enterotoxin and a viral cytotoxin. This impairs cell growth, proliferation, and inhibition of ion transport [39]. In an immune-suppressed setting, infection with opportunistic agents such as *Blastocystis hominis*, *Candida albicans*, and *Cryptosporidium* leads to mucosal injury and diarrhea. HIV directly and indirectly causes malnutrition, immune impairment, and intestinal dysfunction. Antiretroviral therapy may also cause persistent diarrhea [10].

The aforementioned risk factors highlight the importance of recognizing that optimal management of diarrheal episodes is essential to progression to persistent diarrhea. It is natural that given the close relationship between diarrheal disorders, malnutrition, and HIV, persistent diarrhea is widely recognized as a nutritional disorder and that optimal nutritional rehabilitation is considered a cornerstone of its management.

Consequences of Persistent Diarrhea

As diarrhea becomes "persistent," malnutrition becomes increasingly manifest secondary to anorexia and impaired nutrient balance resulting from mucosal injury, malabsorption, and nutrient losses [40]. This sequence is supported by the observation that *Shigella* infection – characterized by intense tissue catabolism and nutrient losses – almost doubles the risk of persistent diarrhea [41], dehydration [3], and why bloody diarrhea (caused by *Shigella* spp. in 45–67% cases and *Campylobacter* in 35–37% cases) so often reported to be a risk factor for persistent episodes [3, 42]. Children with bloody diarrhea are at high risk of morbidity and mortality [3]. The importance of *Shigella* is reflected in the report from at a large hospital center in Bangladesh that the frequency of persistent diarrhea diminished as the isolation rate of *Shigella* decreased between 1991 and 2010. Mucosal injury also explains why by day 14 the manifestations of persistent diarrhea are primarily those of a malabsorption and malnutrition syndrome that requires careful dietary and nutritional management until the mucosal damage is reversed and new normally functioning epithelial cells are regenerated.

There are several reasons why malnutrition should both predispose to and follow persistent diarrhea. These range from achlorhydria with increased risk of small bowel contamination, systemic immune deficiency, intestinal and pancreatic enzyme deficiency, and altered intestinal mucosal repair mechanisms following an infectious insult. An independent relationship has also been demonstrated between cutaneous anergy and the subsequent risk of development of persistent diarrhea [43]. There has been much interest in the possibility that such transient immune deficiency may also be a marker of concomitant micronutrient deficiency [44]. The most striking example of the critical role that the immune system plays in the pathogenesis of persistent diarrhea is the relationship of HIV/AIDS. This is exemplified by the host of studies linking persistent diarrhea with cryptosporidiosis [45] and other parasitic infections [46] in Africa and Asia.

A clear understanding of alterations in intestinal morphology and physiology is crucial toward the development of interventional strategies, but there has been little progress in our understanding of this problem in developing countries. This has been largely due to a paucity of studies formally evaluating intestinal biopsy findings in representative populations. A wide variety of pathological changes has been described after persistent diarrhea, however, ranging from near-normal appearance to mucosal flattening, crypt hypertrophy, and lymphocytic infiltration of the mucosa [47]. Recent elaborate electron microscopic studies of intestinal mucosa in persistent diarrhea reveal patchy villous atrophy and intraepithelial lymphocytic infiltration as well as severe mucosal damage and villous atrophy [48, 49]. Poor intestinal repair is regarded as a key component of the abnormal mucosal morphology. However, the exact factors underlying this ineffective repair process and continuing injury are poorly understood. The end result of this mucosal derangement is reduced absorption of luminal nutrients, as well as increased permeability of the bowel to abnormal dietary or microbial antigens [50]. Alterations of intestinal permeability in early childhood may reflect changes in intestinal mucosal maturation [51] and may be affected by concomitant enteric infections [52].

Management

It is imperative to consider the child's age and clinical manifestations to determine proper treatment in cases of persistent diarrhea. A paucity of diagnostic facilities limits the microbiologic evaluation of diarrhea in many parts of the world. Lack of awareness regarding cow's milk protein allergy and immunodeficiency-associated diarrhea is of par-

Table 17.1 Impact of interventions to prevent and control diarrhea

Intervention	Effect estimates
Water sanitation and hygiene	31–48% risk reduction for diarrhea by handwashing with soap and 31–52% risk reduction for diarrhea with improved water quality
Exclusive breastfeeding for 6 months	165% (10.5 times greater risk of death) increase in diarrhea among 0- to 6-month-old infants if not breastfed Not breastfeeding exclusively results in excessive risk of diarrhea prevalence (RR 2.15–4.90), incidence (RR 1.26–2.65), mortality (RR 2.28–10.52) and all-cause mortality (RR 1.48–14.40) in infants 0–5 months
Adequate complementary feeding among children 6–23 months, including adequate micronutrient intake	6% reduction in all child mortality
Preventive zinc supplementation	23% reduction in diarrhea-related mortality
Preventive vitamin A supplementation	23% reduction in all-cause mortality
Vaccines for rotavirus	74% reduction in very severe rotavirus infection 47% reduction in rotavirus hospitalization
Vaccines for cholera	52% effective against cholera infection
ORS	69% reduction in diarrhea-specific mortality
Prevention of HIV in children	2% reduction in all child mortality
Dietary management of diarrhea	Lactose-free diets reduce the duration of diarrhea treatment failure significantly by 47%
Therapeutic zinc supplementation	66% reduction in diarrhea-specific mortality 23% reduction in diarrhea hospitalization 19% reduction in diarrhea prevalence
Antibiotics for cholera	63% reduction in clinical failure rates 75% reduction in bacteriological failure rates
Antibiotics for *Shigella*	82% reduction in clinical failure 96% reduction in bacteriological failure rates
Antibiotics for cryptosporidiosis	52% reduction in clinical failure rates 38% reduction in parasitological failure rates
Community-based intervention platforms for prevention	160% increase in the use of ORS 80% increase in the use of zinc in diarrhea 76% decline in the use of antibiotics for diarrhea
Community case management (CCM)	CCM of diarrhea with zinc and ORS reduced diarrheal deaths among children under the age of 5 years by 93%
Financial support schemes	Conditional transfer programs: 14% increase in preventive healthcare use, 22% increase in the percentage of newborns receiving colostrum, and 16% increase in the coverage of vitamin A supplementation

ticular concern. Optimal prevention and management of acute diarrheal illnesses are the ideal strategies to prevent persistent diarrhea. Treatment is focused on reversing dehydration (if present), nutritional interventions including balanced protein energy, pancreatic enzyme replacement therapy (PERT), micronutrient supplements, and judicious use of antibiotics for certain types of inflammatory diarrhea. Oral rehydration solutions, micronutrient supplementation, algorithm-based diet regimens, and good supportive care are sufficient in most children above 6 months of age with persistent diarrhea (Table 17.1).

Rapid Resuscitation, Antibiotic Therapy, and Stabilization

Most children with persistent diarrhea and associated malnutrition are not severely dehydrated and oral rehydration may be adequate. Indeed, routine use of intravenous fluids in severe acute malnutrition should be avoided; acute severe dehydration and associated vomiting may require brief periods of intravenous rehydration with Ringer's lactate. Acute electrolyte imbalance such as hypokalemia and severe acidosis may require correction. More importantly, associated systemic infections (bacteremia, pneumonia, and urinary tract infection) are well-recognized complications of severe acute malnutrition in children with persistent diarrhea and a frequent cause of early mortality. Almost 30–50% of malnourished children with persistent diarrhea may have an associated systemic infection requiring resuscitation and antimicrobial therapy [53, 54]. Children with bloody diarrhea (caused by *Campylobacter*, *Shigella*, or some parasites) require antibiotic therapy for enteric pathogens. Ceftriaxone, ciprofloxacin, and pivmecillinam have shown to be effective against local strains of *Shigella* [55], whereas azithromycin and fluoroquinolones are highly effective against *Campylobacter* gastroenteritis. Treatment against strain of *Shigella* is recommended to be changed if no improvement is observed within two days of treatment.

Emergence of antibiotic resistance strains of enteric pathogens are a great threat worldwide. For example, multidrug resistance has been reported against *Shigella* sp. in Ethiopia [56], Nigeria [57], and Mozambique [58]. In such instances, fortified food therapy (such as zinc and iron), immunotherapy [59–61], lactose-free diet [62], fecal microdata transplantation (used in *Clostridium difficile*-associated diarrhea) [63], and probiotics [64–66] are the alternative treatment options against the enteric pathogens. It should be emphasized that there is little role for oral antibiotics in persistent diarrhea as in most cases the original bacterial infection triggering the prolonged diarrhea has disappeared by the time the child presents. Possible exceptions are appropriate treatment for dysentery [67] and adjunctive therapy for cryptosporidiosis in children with HIV and persistent diarrhea [68].

Oral Rehydration Therapy

Death by persistent diarrhea is usually caused by malnutrition or by hypovolemia (dehydration). It is over 40 years since the efficacy of oral rehydration therapy (ORT) was clearly demonstrated, following discovery of glucose-stimulated sodium uptake by intestinal villus cells [62, 69, 70]. This is the preferred mode of rehydration and replacement of on-going losses. The net effect is expansion of the intravascular compartment and rehydration, usually sufficient for all but the most severely dehydrated patients require initial intravenous fluids [71]. World Health Organization (WHO) has recommended a new low osmolarity solution for the management of diarrhea [72], which contains 75 mmol/L glucose, 75 mEq/L sodium, and 20 mEq/L potassium, at an osmolarity of 245 mOsm/L. Potassium chloride can also be added in ORS solution (to provide 40 mEq/L of potassium) for severely malnourished children with depleted potassium levels. In general, the standard WHO oral rehydration solution is adequate, while recent evidence indicates that hypo-osmolar rehydration fluids [73] as well as cereal-based oral rehydration fluids may be advantageous in malnourished children. A number of modifications have been proposed, for example, cereal (rice)-based ORT, addition of certain amino acids (glycine, alanine, or glutamine) to further increase sodium absorption and/or hasten intestinal repair, or supplementation with zinc, but none have been shown to be consistently superior to low osmolality ORS [74, 75]. However, in Nigeria, homemade fluids and cereal-based oral therapies have proven to be effective in the management of diarrhea [76].

Enteral Feeding

Nutritional rehabilitation can break the vicious cycle of chronic diarrhea and malnutrition and is considered the cornerstone of treatment. It is exceedingly rare to find persistent diarrhea in exclusively breastfed infants, and with the exception of situations where persistent diarrhea accompanies perinatally acquired HIV infection, breastfeeding must be continued. Although children with persistent diarrhea may not be lactose intolerant, administration of a lactose load exceeding 5 g/kg/day may be associated with higher purging rates and treatment failure. Alternative strategies for reducing the lactose load while feeding malnourished children who have prolonged diarrhea include addition of milk to cereals such as rice and noodles and replacement of milk with fermented milk products such as yogurt. Lactose-free diet and low-sucrose and -carbohydrate diet has also been effective in minority of the cases. Mattos et al. [77] claimed that yogurt-based diet is recommended as the first choice for the nutritional management of a mild to moderate persistent diarrhea. A cheap and an easily available yogurt-based diet can be used in mild chronic diarrhea illness of uncomplicated and without enteropathy. Bhutta et al. [36] suggested algorithm for the diagnosis and management of persistent diarrhea (Fig. 17.1). Elimination diet is considered when allergic enteropathy is induced by a cow's milk protein or soy protein [78]. Rarely, when dietary intolerance precludes the administration of cow's milk-based formulations or milk, it may be necessary to administer specialized milk-free diets such as a comminuted or blended chicken-based diet or an elemental formulation. Although effective in some settings, the latter are unaffordable in most developing countries. In addition to rice-lentil formulations, the addition of green banana or pectin to the diet has also been shown to be effective in the treatment of persistent diarrhea [79, 80]. Among children in low- and middle-income countries, where the dual burden of diarrhea and malnutrition is greatest and where access to proprietary formulas and specialized ingredients is limited, the use of locally available age-appropriate foods should be promoted for the majority of diarrhea cases. Nutritionally complete diets comprising locally available ingredients can be used at least as effectively as commercial preparations or specialized ingredients. The usual energy density of any diet used for the therapy of persistent diarrhea should be around 1 kcal/g, aiming to provide an energy intake of a minimum 100 kcal/kg/day, and a protein intake of between 2 and 3 g/kg/day. Additionally, potassium should also be included in the diet providing approximately 5 mEq/kg per day. In selected circumstances when adequate intake of energy-dense food is problematic, the addition of amylase to the diet through germination techniques may also be helpful. Recent WHO guidelines recommend that children with severe acute malnutrition who present with either acute or persistent diarrhea can be given ready-to-use therapeutic food (RUTF) in the same way as children without diarrhea, whether they are being managed as inpatients or outpatients. And these chil-

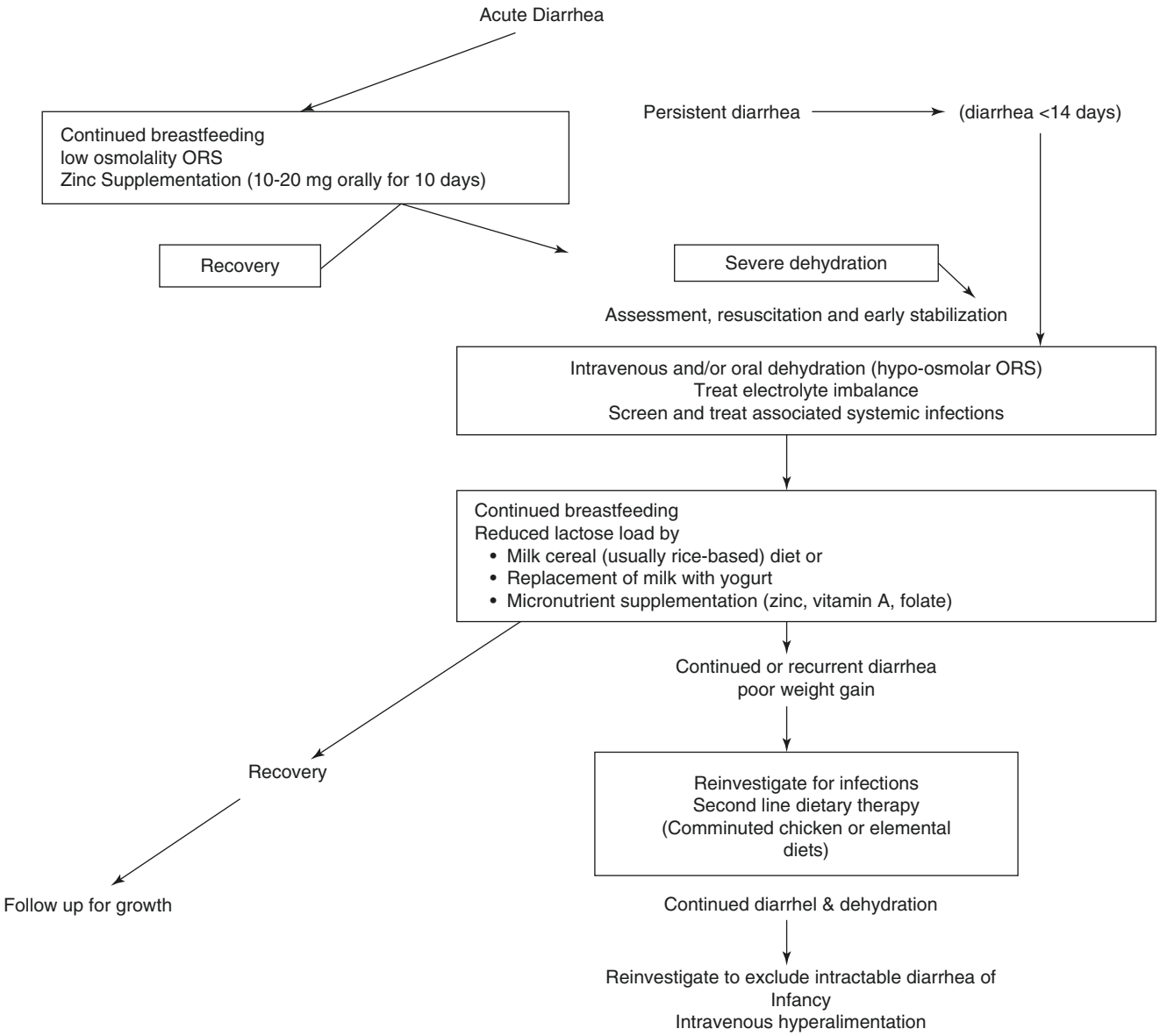

Fig. 17.1 Algorithm of diagnosis and management of persistent diarrhea

dren with severe acute malnutrition (SAM) are typically managed by a course of broad-spectrum antibiotics with a gradual increase in full caloric intake. Hypophosphatemia is common during the refeeding syndrome which is mitigated by providing phosphorus-rich food such as milk-based feed to the children [3].

Micronutrient Supplementation

It is now widely recognized that most malnourished children with persistent diarrhea have associated deficiencies of micronutrients including copper, folic acid, zinc, iron, vitamin A, and minerals [81]. This may be a consequence of poor intake and continued enteral losses and requires nutritional rehabilitation [82]. While the evidence supporting zinc administration in children with persistent diarrhea is persuasive, it is likely that these children have multiple micronutrient deficiencies. Concomitant vitamin A administration to children with persistent diarrhea has been shown to improve outcome [83, 84], especially in HIV endemic areas [85]. In Bangladesh, a combination of vitamin A, zinc, and ORS has proven to be effective at reducing child mortality caused by diarrhea [86]. WHO recommended 10 mg/day of zinc for 10–14 days for children <6 months and 20 mg/day for children >6 months [87]. Zinc has shown a significant effect among children >6 months with shortened duration of persistent diarrhea by approximately 16 hours [88, 89]. However,

zinc decreases the absorption of copper and causes copper depletion in the body [89]. While the association of significant anemia with persistent diarrhea is well recognized, iron replacement therapy is best initiated only after recovery from diarrhea has started and the diet is well tolerated. Thus, in July 2019, WHO has recommended to administer folate, iron, vitamin A, copper, and magnesium twice a day for two weeks [90].

Antidiarrheal Drugs

Antidiarrheal drugs are not recommended due to their inefficiency, side effects, and possibly also as causing prolonged release of enteric pathogens. Similarly, antiemetic drugs are also not encouraged because of the sedation caused by them which may interfere with ORT.

Pancreatic Enzyme Replacement Therapy (PERT)

Persistent diarrhea causes pancreatic exocrine insufficiency due to decreased stimulation to pancreas caused by prolonged mucosal injury. PERT prescribed in conjunction to regular treatment has proved to be beneficial in replacing pancreatic enzyme deficiency. Studies from Indonesia identified children (6–60 months) with pancreatic enzyme deficiency through fecal elastase-1 test [91, 92]. These children were provided with PERT (8371 United States Pharmacopeia [USP] units of lipase) three times a day for a month. This therapy showed decrease in duration of diarrhea by 3–7 days in the intervention group when compared to the placebo group.

Improved Case Management of Diarrhea

Improved management of diarrhea through prompt identification and appropriate therapy significantly reduces diarrhea duration, its nutritional penalty, and risk of death in childhood. Improved management of acute diarrhea is a key factor in reducing the burden of prolonged episodes and persistent diarrhea. The WHO/UNICEF recommendations to use low-osmolality ORS and zinc supplementation for the management of diarrhea, coupled with selective and appropriate use of antibiotics, have the potential to reduce the number of diarrheal deaths among children through Community Case Management (CCM) and Integrated Management of Childhood Illness (IMCI). Community-based interventions to diagnose and treat childhood diarrhea through community health workers leads to a significant rise in care-seeking behaviors for diarrhea and are associated with significantly increased use of ORS and zinc at household level as well as reduction in the unnecessary use of antibiotics for diarrhea by 75% [93].

Other Potential Modalities

The factors associated with persistent diarrhea are small intestinal mucosa injury, persisting infective colonization, and bacterial particles and toxins that are translocated into the host cell and downregulated host immune system. These circumstances alter interrelation between the normal flora and the host, which can worsen prolonged inflammation. The rationale for using probiotics in the treatment of persistent diarrhea lies in their ability to survive and reproduce in the host's gut and in their proven role in the treatment of acute diarrhea. Recent evidence suggests modest effect of probiotics with reduced duration of persistent diarrhea and stool frequency [94]. Because of probiotics known immunomodulatory effect and very significant mortality and morbidity rate from persistent diarrhea in developing countries, it is imperative to highlight the necessity for well-designed studies to define the role of probiotics in persistent diarrhea.

Follow-Up and Nutritional Rehabilitation in Community Settings

Given the high rates of relapse in most children with persistent diarrhea, it is important to address the underlying risk factors and institute preventive measures. These include appropriate feeding (breastfeeding, complementary feeding) and close attention to environmental hygiene and sanitation. This poses a considerable challenge in communities deprived of basic necessities such as clean water and sewage disposal.

In addition to the preventive aspects, the challenge in most settings is to develop and sustain a form of dietary therapy using inexpensive, home-available, and culturally acceptable ingredients which can be used to manage children with persistent diarrhea. Given that the majority of cases of persistent diarrhea occur in the community and that parents are frequently hesitant to seek institutional help, there is a need to develop and implement inexpensive and practical home-based therapeutic measures [95] and evidence indicates that it may be entirely feasible to do so in community settings [96, 97].

Conclusions

Persistent diarrhea remains an existential threat, especially among children under 5 years of age in resource-limited countries where quality of life and access to health care are low.

Persistent diarrhea in children contributes to childhood malnutrition and mortality. Most of the knowledge and tools (bioinformatics, metagenomics) needed to prevent diarrhea-associated mortality in developing countries, and especially persistent diarrhea, are available. These require concerted and sustained implementation in public health programs. Given the emerging evidence of the long-term impact of childhood diarrhea on developmental outcomes [98], it is imperative that due emphasis is placed on prompt recognition and appropriate management of persistent diarrhea besides the focus on improving child nutrition and hygiene.

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HIV and the Intestine

18

Andrea Lo Vecchio and Francesca Wanda Basile

Introduction

The pandemic spread of HIV has been reversed over the last three decades, and in 2015, the United Nations embraced the ambitious target to end the AIDS epidemic by 2030 as a part of the Sustainable Development Goals [1].

The introduction of successful interventions for a timely diagnosis, together with the advancements in pediatric antiretroviral treatment (ART), are shaping the natural history of HIV infection in childhood, which is evolving into a chronic disease, the control of which strictly depends on accessibility and adherence to ART [2].

ART has dramatically changed the spectrum of HIV-associated complications, but the gastrointestinal (GI) tract remains a major target, especially in resource-limited countries where a significant proportion of children are still present with features of advanced disease; at this stage, GI symptoms and failure to thrive are frequent, as they reflect the combined effects of opportunistic infections, HIV enteropathy, and immune impairment. In this scenario, healthcare costs, morbidity, and mortality are substantially increased, particularly for those children with severe malabsorption and malnutrition requiring repeated hospitalizations. In many such cases, a multidisciplinary approach involving specialists in gastroenterology, HIV/infectious diseases, nutrition, surgery, and microbiology is needed (Fig. 18.1).

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The Spectrum of GI Disorders in HIV Infection

The GI system is a topographical target of HIV infection and ART. Symptoms can involve any tract of the GI from the oral cavity to the large intestine, liver, and pancreas. This paragraph provides an overview of the GI involvement in HIV, and topics are discussed in detail in the specific sections.

In the ART era, many of the common HIV-related disorders have vanished but they can still be observed in children who are failing on treatment or who access the healthcare system already with features of advanced disease.

For this reason, the approach to GI symptoms in any HIV-infected children with adequate virological and immunological control should parallel that of any other non-HIV patient living with a chronic health condition. In this scenario, drug history is the first step to rule out any specific ART side effects, as transient drug-induced GI symptoms like abdominal distension, nausea, and diarrhea are common, particularly in the first year of ART. Up to 29% of patients on any class of antiretrovirals experience mild-to-moderate diarrhea. Pancreatitis and hepatitis are infrequent but are potentially severe complications associated with the use of antiretrovirals.

Table 18.1 summarizes ART-associated GI and hepatic adverse events [3–11].

Intestinal dysfunction is a specific syndrome almost inevitably seen in children at advanced stages, which has the features of a malabsorption disorder. Clinical manifestations may be limited or absent and not necessarily associated with severe diarrhea. As a consequence of the reduction in the intestinal absorptive surface and increased permeability, the predominant features are steatorrhea, micronutrient deficiencies, and progressive failure to thrive. The absence of any GI pathogen, despite extensive microbiological investigation, defines HIV enteropathy [12].

In patients with advanced HIV disease, the stage of immunodeficiency, reflected by the age-specific CD4+ lymphocyte

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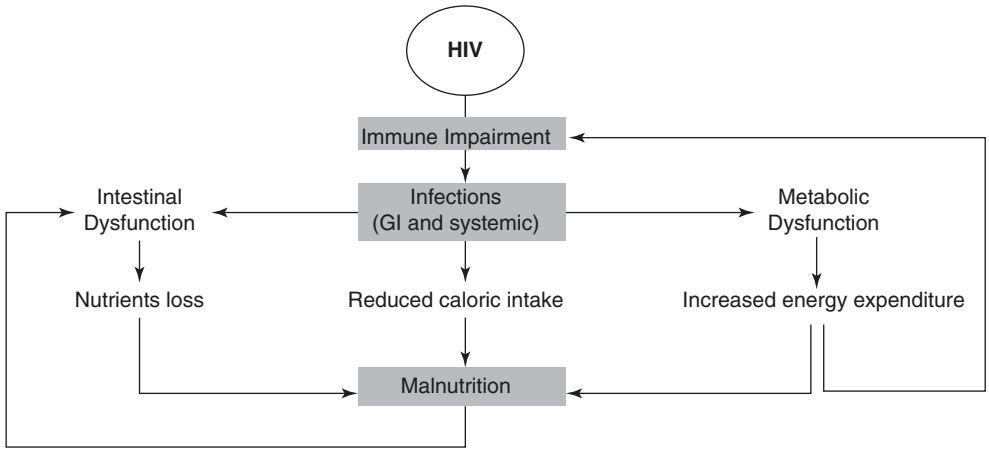

Fig. 18.1 Pathway of malnutrition and gastrointestinal disorders in children with HIV infection. A complex interplay exists among these conditions

Table 18.1 ART-associated GI and hepatic adverse events

Adverse event	ART	Incidence	Clinical characteristics	Management
Nausea/vomiting	Protease inhibitors and all ARV	<15%	Early onset, usually improves within 2 months since the beginning of ART Nausea can present with or without vomiting and abdominal discomfort	Reassure patient and recommend taking protease inhibitor with food If persistent emesis, consider switching to a different regimen (with smaller tablet size)
Diarrhea	Protease inhibitors and all ARV	Up to 29%	Early onset, usually improves within 2 months Nonbloody, and usually nonwatery, mild-to-moderate diarrhea Some FDC tablets contain lactose that may trigger diarrhea in patients with lactose intolerance	Rule out infections and other organic causes of persistent/chronic diarrhea Reassure patient. Dietary modifications may help. Treat dehydration if present If persistent/severe symptoms, consider switching to a different regimen
Drug-induced liver disease (idiosyncratic hepatotoxicity, hypersensitivity, and hyperbilirubinemia)	*Hepatitis:* all ARV (strong association with nevirapine and efavirenz) *Indirect hyperbilirubinemia:* atazanavir *Hepatic steatosis:* zidovudine	1–9% (severe hepatitis and hypersensitivity reaction <1%)	*Hepatitis, hyperbilirubinemia:* early onset, usually in the first months of therapy *Steatosis:* develops after years The clinical scenario of liver disease ranges from asymptomatic hypertransaminasemia to severe hepatitis with lactic acidemia and rash in hypersensitivity reactions ATV-associated hyperbilirubinemia is usually an isolated finding with normal transaminases	Rule out infections (HAV, HBV, HCV, EBV and CMV) Asymptomatic hypertransaminasemia: treatment discontinuation, if ALT < 5 × UNL Symptomatic hepatitis: stop all ART and other potentially hepatotoxic drugs. If on nevirapine, permanently discontinue Isolated hyperbilirubinemia does not require atazanavir discontinuation
Pancreatitis	NRTIs and protease inhibitors	<2%	Can develop any time, usually after months of ART Acute pancreatitis with significantly raised serum lipase and amylase	Stop the offending drug and do not reintroduce Symptomatic management

count, should always be looked at because frequency (and the etiology) of opportunistic infections (OI) is related to the CD4 count and rises exponentially with lower CD4+ counts. Intestinal infections are a major cause of morbidity and mortality, especially for children, and where the availability of ART is limited. Candidiasis, the most common HIV-related fungal infection, usually involves mouth and throat. Cryptosporidium and mycobacterium avium complex (MAC) are common GI opportunistic pathogens in developing countries, while, in high-income settings, CMV prevails. Of note, although, in the ART era, the number of cases of diarrhea by OI has fallen dramatically worldwide, the total number of patients experiencing diarrhea has changed very little, probably as a consequence of the global increase in GI diseases (infectious and noninfectious) unrelated to immunodeficiency [13].

HIV-associated malignancies, like Kaposi sarcoma and non-Hodgkin B-cell lymphomas, often affect the GI tract and may present with symptoms like diarrhea, rectal bleeding, or acute abdomen.

Overt hepatic disease is not frequent in HIV-infected children, but isolated hepatomegaly or mild hypertransaminasemia is a common finding often associated with nutritional deficiencies, nonalcoholic fatty liver disease (NAFLD), medication-associated liver injury, or coinfection with chronic viral hepatitis (HBV or HCV), which should always be ruled out [14].

Exocrine pancreas impairment, although rare in children on ART, may be considered as a component of the HIV-associated digestive dysfunction, and the diagnostic workup of HIV-related diarrhea should include measurements of fecal pancreatic enzymes [15].

In resource-limited countries, undernutrition is a frequent comorbidity in HIV-infected children who present late and malnutrition enteropathy can precipitate intestinal dysfunction; it is often impossible to understand to what extent undernutrition is the consequence of primary food insecurity, HIV enteropathy, or OIs. In the majority of cases, it is likely to be a multifactorial event that contributes significantly to the morbidity and mortality of children with advanced HIV disease [16].

In conclusion, the intestinal involvement in HIV infection is closely related to access to ART and shows a geo-economical pattern. In high burden settings with limited access to health services, intestinal OIs are still a major problem, and there is a complex interplay among malabsorption, immune impairment, and undernutrition. In children receiving ART, there is a residual intestinal dysfunction, with no or limited symptoms specific to HIV infection or treatment.

Pathophysiology of HIV-Associated Intestinal Damage

Since the discovery of HIV as the causative agent of AIDS, histological abnormalities of the GI tract were observed, and the term "HIV enteropathy" was first used in 1984. HIV enteropathy was classically diagnosed in AIDS patients with symptoms of refractory intestinal dysfunction when other etiologies have been ruled out [17].

Enterocyte damage and mucosal atrophy have become the histological hallmark of HIV enteropathy, but other common findings include inflammatory infiltrates of lymphocytes, crypt hyperplasia, and villous blunting, in the absence of detectable enteric pathogens [18].

In recent years, there have been great advancements in the understanding of this condition, and the scientific discoveries have shed light on the complex mosaic of interactions between HIV and gut. HIV does primarily target gut-associated lymphoid tissue (GALT) but has also shown the ability to affect the intestinal barrier at all levels, via direct and indirect mechanisms.

HIV-associated intestinal damage begins at the early stages of the infection. The clinical spectrum of the disease encompasses a variety of manifestations ranging from a subclinical impairment of the mucosal barrier function (when effective ART is promptly initiated) to the "classical" picture of chronic intestinal failure in advanced HIV disease characterized by increased intestinal permeability, bile acid and vitamins malabsorption, lactose intolerance, protein-losing enteropathy, and failure to thrive [19, 20].

The damage to mucosal immunity is early and somehow irreversible. Antiretroviral therapy, although started immediately, can only partially counteract it. This aspect of HIV-associated intestinal damage has acquired increasing importance due to its role in microbial translocation, and therefore, as a contributing factor to the chronic inflammation state that is characteristic of people living with HIV despite good virologic response [21–23].

In the early phases of the acute infection, GALT becomes a site of intense viral replication and CD4+ T-cell destruction. Activated memory T cells expressing C-C chemokine receptor type 5 (CCR5) are largely represented in the gut and constitute a preferential target for HIV. More than half of the GI CD4+ cells are infected by the virus, meaning that the intestine constitutes an important viral reservoir [24, 25].

Another mechanism of intestinal immune barrier damage involves the ability of the virus to elicit a local response that leads to an increase in proinflammatory cytokines (IL-6, IL-10, and IFN-γ) in the lamina propria, similar to the pathogenesis of GI damage in inflammatory bowel diseases [26]. The inflammatory cytokines, particularly IFN-γ, are also responsible for the depletion of mucosal Th17, a key population of CD4+ T cells in maintaining gut immune homeostasis. Moreover, HIV exerts a direct cytotoxic effect on this CD4 subset; thus, acting synergistically. Th17 produces the interleukin IL-17, which together with IL-22 produced by Th22 have a pivotal role in blocking microbial translocation from the gut lumen [27, 28].

The complex interplay between HIV and GI is not limited to its damage to the immune system.

A direct "virotoxic" effect on the intestinal barrier has been hypothesized for the first time more than 20 years ago [29]. There is convincing evidence that the virus acts both at the cellular (enhancing enterocyte apoptosis) and paracellular level (disrupting tight and adherens junctions). As a consequence, the intestinal wall becomes leaky and this structural loss further contributes to microbial translocation [30].

Intestinal epithelial apoptosis is an early event during HIV infection [31]. It has been demonstrated that the intracellular imbalance between antioxidant defenses and reactive oxygen species (ROS), in favor of the latter, is involved in enterocyte apoptosis [32]. Tat, a transactivating peptide secreted by HIV-infected cells and essential for viral replica-

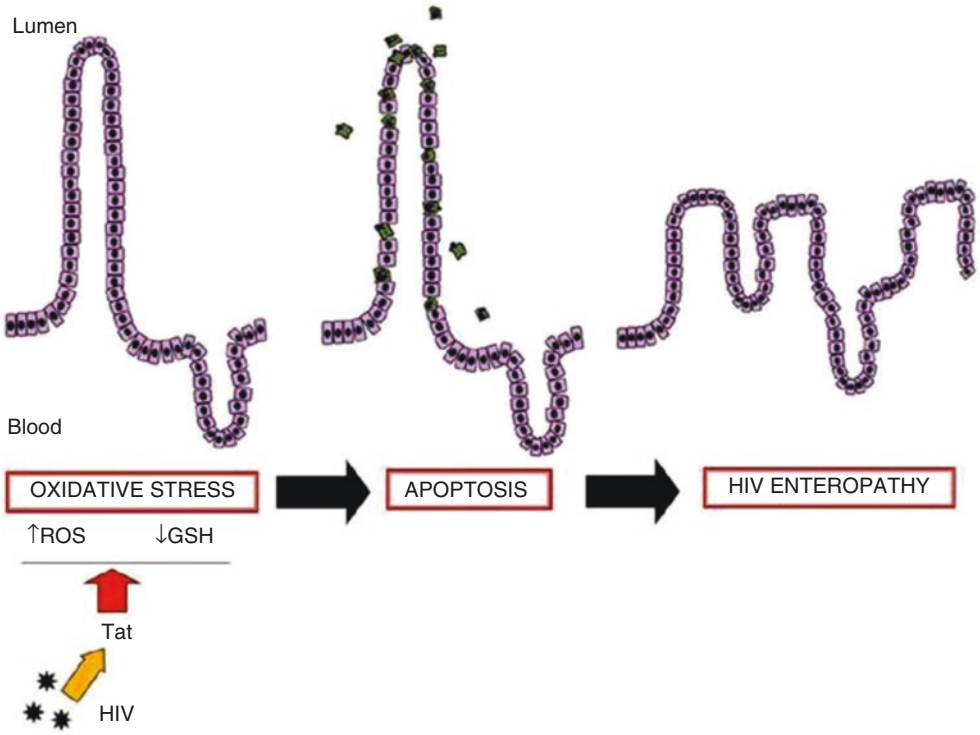

Fig. 18.2 Schematic representation of the mechanism of HIV Tat-induced intestinal epithelium damage. Tat triggers oxidative stress by increasing the ROS intracellular level and affecting the GSH/GSSG ratio. This leads to programmed cell death (apoptosis) and epithelial damage. Together with altered ion secretion and glucose transport, these steps could represent key mechanisms in HIV enteropathy. ROS reactive oxygen species, GSH glutathione, Tat transactivator factor, HIV human immunodeficiency virus. (Reprinted from Ref. [33] with permission)

tion, has been identified as a key mediator in this process (Fig. 18.2).

In support of the role of Tat via an oxidative stress-mediated pathway, increased epithelial apoptosis has been observed in ex vivo jejunal specimens from HIV-positive patients at baseline and from HIV-negative specimens exposed to Tat [33, 34]. In addition to the ROS-mediated mechanism, several interleukins (e.g., IL-18, IL-6, and IL-8) produced by the enterocytes during the infection, play a role in increased intestinal permeability through the downregulation of the macromolecules that form both tight and adherens junctions [34, 35].

Gut Microbiota in HIV Infection

The intestinal microbiota is a key component at the frontline of the gut barrier. Its diversity is physiologically influenced by multiple factors including age, sex, diet, and sexual behaviors. In recent years, research has focused on the impact of HIV infection on gut microbial community composition and function.

Increasing evidence suggests that the dysbiosis observed in people living with HIV is associated with increased circulating markers of microbial translocation and systemic inflammation, such as IL-6, lipopolysaccharide (LPS), and soluble CD14 (sCD14), in both adults and children [36].

HIV affects the balance of commensal bacteria, increasing the proportion of pathobionts (commensal bacteria that have harmful potential in a permissive context) [37].

Overall, the most common finding at the phyla level is the increase in Proteobacteria and a decrease in Firmicutes, which include *lactobacilli, streptococci, staphylococci,* and *clostridia*. More specific alterations within the phyla involve the shift from *Bacteroides* to *Prevotella* spp. (Bacteroidetes), and the increase in members of the family Enterobacteriaceae such as *Escherichia, Klebsiella* and *Yersinia* spp. Pathobionts are usually associated with a proinflammatory condition, and in vitro experiments have demonstrated that some species may act in synergy with HIV, boosting the secretion of IL-10 and TNF-a from infected peripheral mononuclear cells [38–40].

Gut microbiota research has just recently focused on perinatally infected children, who exhibit lower alpha diversity (species richness) and increased beta diversity (interindividual diversity) compared with uninfected children. The findings from a Zimbabwean study are similar to the findings in the adult population and show an abundance of *Prevotella* and *Corynebacterium* at the genus level. Moreover, children with severe immunosuppression (CD4 $\leq$ 400/mmc) have enriched Enterobacteriaceae. Such family is frequently involved in microbial translocation, but the clinical implications of this finding need to be investigated further. Notably, the results from the Zimbabwean

cohort are in line with a similar study that examined the gut microbiome of perinatally infected children from a high-income country. However, it has to be noted that some minor discrepancies exist regarding the relative abundance of specific taxonomic groups [41, 42].

Also HIV-exposed (but uninfected) infants exhibit a different gut microbiota from unexposed children, which is characterized by an abundance of the taxa Lachnospiraceae. Such difference does not seem to be pathogenic per se, as judged by normal microbial translocation and inflammation markers in these infants. Results are slightly different between studies according to the main nutritional source (breast milk vs. formula feeds) [43, 44].

As seen with other manifestations of the HIV-induced intestinal damage, long-course ART can counteract gut dysbiosis. Perinatally infected children show a restored microbiota (alpha diversity) after more than 10 years on treatment. ART duration, however, does not seem to impact on beta diversity [42, 45].

A variety of drugs can affect gut microbial composition, but little is known about the direct effect of antiretroviral drugs. It is a shared opinion that antiretrovirals have a distinct impact on the intestinal microbiota, but to date the quality of existing studies is undermined by several confounding factors. It appears that regimens based on a protease inhibitor have the most harmful effect [46].

Cotrimoxazole, commonly prescribed for prophylactic use in HIV-infected children, does not modify the microbial composition and exhibits an antibiotic effect on gut resident *Streptococci* that reduces intestinal and systemic inflammation [33].

Intestinal Barrier Damage Response to ART and Novel Strategies

Intestinal dysfunction, if present, improves shortly after initiation of ART, in parallel with the immune reconstitution in blood and a decrease in viral load which is seen both in blood and rectal mucosa [29].

The beneficial effects of therapy on GALT are not able to fully revert the damage caused by HIV in the initial phases of the infection, and subclinical impairment of the GI immune and barrier function has been documented in up to one-third of HIV-infected patients on treatment, regardless of the ART regimen [47].

Such damage, associated with intestinal dysbiosis, creates a permissive environment for microbial translocation, which has emerged as a driver for the persistent systemic inflammation seen in people living with HIV.

However, it has to be noted that the majority of the results are based on cohorts of ART-experienced adults and teenagers with the most diverse backgrounds and ART histories; only in recent times, the research has been focusing on groups of "tested and treated" individuals, where an early start of the antiretroviral treatment may further decrease the proviral reservoir in the GALT and possibly reduce the long-term consequences of the intestinal damage [22, 48].

Recent evidence from pediatric studies supports this hypothesis and downsizes the role of microbial translocation. Irrespective of the relative abundance of HIV-susceptible memory CD4+ T cells in the gut compared with adults, perinatally HIV-infected infants started on a very early ART have a smaller proviral reservoir in GALT and progressively lower levels of chronic inflammation and microbial translocation biomarkers; confirming the hypothesis that prompt therapeutical intervention in the acute phases of the infection is effective in limiting the intestinal damage [49–51].

In parallel, research has focused on additional strategies to modulate dysbiosis, like the supplementation with probiotics and prebiotics. Prolonged use of probiotics is associated with an increase in CD4+ count and Th17 subset in GALT and peripheral blood, reduction in inflammation markers and T-cell activation, and recovery of gut barrier integrity. Prebiotic mixtures are also promising modulators of the immune function and gut microbiota composition. A multicenter randomized controlled trial in South Africa showed that the administration of a prebiotic/probiotic combination induced a strong bifidogenic effect on healthy HIV-exposed infants, but more data from controlled trials to endorse the routine use of probiotics in the pediatric HIV population are needed [52–55].

Upper Gastrointestinal Symptoms

Patients with HIV/AIDS, either on ART or not, frequently complain about symptoms related to upper gastrointestinal tract involvement (oral cavity, esophagus, and stomach).

Oral thrush, dysphagia, difficulty swallowing, or odynophagia may be secondary to opportunistic infections and are reported by naïve or not compliant children and adolescents, or by those who are failing ART. Feeding refusal and weight loss may be the only signs in neonates and infants.

Upper GI opportunistic infections, including Candida species, herpes simplex virus, and cytomegalovirus, are often the underlying cause of such symptoms, and their likelihood is related to the degree of immunosuppression, resulting quite common in subjects with a CD4+ count <200 cells/ul.

Thrush, appearing white-yellow, hard-to-remove plaques due to Candida infection, is a characteristic sign in young HIV/AIDS infants and children. Since oropharyngeal candidiasis and esophagitis are concomitant in 75% of cases, an upper GI endoscopy is mandatory in all severely immunosuppressed children with oral thrush to exclude distal esoph-

agus involvement, especially in patients who show loss of appetite, dysphagia, fever, or weight loss [56]. A history of oropharyngeal candidiasis or antibiotic administration (either prophylactic or therapeutic) is virtually always present in children with esophagitis. Most severe Candida infections can cause necrotizing esophagitis, bleeding, and, occasionally, esophagus perforation.

In the pre-ART era, Candida was by far the first cause of esophageal complaints, and empirical treatment with fluconazole—instead of endoscopy—was considered as the best strategy, especially in patients with oral thrush. With endoscopy being more routinely performed in HIV-infected patients, causes of esophagitis other than Candida have emerged: CMV, HSV, and idiopathic esophageal ulcers in particular. CMV esophagitis may result in severe ulceration or stenosis. Idiopathic esophageal ulcers are probably immunologically mediated, caused by HIV itself [57, 58].

Patients with HIV are more likely to have hypochlorhydria, although the reasons are still unclear. This condition predisposes to GI infections but seems to reduce the incidence of symptoms related to esophageal reflux or peptic ulceration compared to uninfected subjects. This association seems to be true also in low- to middle-income settings, despite the high prevalence of *Helicobacter pylori*. Surprisingly, the hypochlorhydria appears to be reversed by antiretroviral therapy, and peptic ulceration (and gastroesophageal reflux) may increase in HIV-infected populations receiving ART [16].

Subjects receiving ART frequently complain of nausea, dyspepsia, anorexia, or vomiting as a common side effect of antiretroviral drugs (mainly old-generation PI) [59]. Empirical treatment with proton-pump inhibitors has to be considered on a case-by-case basis in HIV-infected patients because these drugs could reduce the oral absorption of selected protease inhibitors [60]. Atypical presentation of opportunistic infections should always be considered in this population and upper endoscopy is recommended for differential diagnosis if symptoms persist. Multiple biopsies are needed since macroscopic normal appearing mucosa does not exclude opportunistic infections. However, less than 2% of adults complaining of dyspepsia and undergoing ART receive a diagnosis of opportunistic infections, with CMV as the most prevalent opportunistic agent [61]. Chronic nonspecific gastritis (mononuclear cell infiltrate) is the most common diagnosis in patients presenting with these symptoms.

Lower Gastrointestinal Symptoms

HIV infection and diarrhea are closely connected in a vicious circle encompassing undernutrition, malabsorption, impairment of immune response, and deterioration of the clinical conditions.

Diarrhea has been reported in as many as 90% HIV-positive children in Africa and 40–80% of those living in high-income countries in the pre-ART era. Parallelly, up to 25% of children accessing hospitals for diarrhea turn to be HIV infected in high-prevalence settings.

HIV infection increases the risk of diarrhea, worsening the symptoms and prolonging its duration. In a recent large study in Mozambique, among 2000 children seeking care for diarrhea, HIV-infected children showed a fivefold increased risk of moderate-to-severe diarrhea, were more frequently hospitalized, and more likely malnourished, stunted, or wasted. In this population, a seven- to tenfold higher mortality rate was reported compared to uninfected subjects presenting either with moderate-to-severe (34 vs. 5/1000 person-weeks at risk) or less severe diarrhea (5 vs. 0.5/1000 person-weeks at risk) [62].

Chronic diarrhea is a hallmark of advanced HIV disease, and several intestinal pathogens, including *Salmonella*, CMV, *Cryptosporidium*, *Mycobacterium*, and *Isospora*, are responsible for AIDS-defining conditions. However, chronic diarrhea may have multiple causes in this population, including HIV-induced enteropathy, small intestinal bacterial overgrowth, malnutrition, and antiretroviral treatment itself.

In underprivileged areas, HIV enteropathy and environmental enteropathy may overlap and the two conditions may be morphologically and functionally indistinguishable with subtle consequences, including malabsorption of micronutrients and drugs, bacterial translocation, and impaired responses to vaccines.

In the era of widespread ART, the etiology and severity of diarrhea vary significantly according to accessibility and adherence to antiretroviral treatment.

Table 18.2 reports a list of the commonest agents in HIV/AIDS children. The likelihood of opportunistic infections is linked to the severity of immunodeficiency, and 200 CD4+ cell/ul seems to be a quite reliable threshold exposing to higher or lower risk of opportunistic agents.

Cryptosporidium, *Giardia*, *Mycobacterium avium*, *Cytomegalovirus*, and ETEC have recently been confirmed as main agents of chronic diarrhea in treatment-naïve children. In children under treatment, common enteric pathogens, including viruses, or other causes of chronic diarrhea should be investigated.

Table 18.2 Main agents causing diarrhea in HIV-infected children

Bacteria	EPEC, Shigella, Salmonella, Listeria, and Mycobacteria
Viral agents	Norovirus, Cytomegalovirus, Herpes simplex, Bocavirus, Rotavirus, Adenovirus, Aichi virus, and Astrovirus.
Parasites	Cryptosporidia, Giardia, Isospora, Hookworm, Strogyloides stercoralis, and Hymenolepis nana
Fungi	Microsporidia (i.e., *Enterocytozoon bieneusi)*, Histoplasma, Candida, and Cryptococcus

Bacterial Diarrhea

The overall incidence of bacterial diarrhea in HIV-infected treatment-naïve adults is at least 100-fold greater than in the general population, and infections in HIV-infected subjects tend to be more protracted and severe than in immunocompetent individuals [63].

The rate of single bacteria found in stools of HIV-infected patients depends on the settings (rural vs. urban and high vs. low income) and the diagnostic methodology.

Campylobacter jejuni and *Salmonella* are frequently isolated in diarrheic stools of children with or without HIV infection. Both infections are usually self-limiting in otherwise healthy children, and do not require specific antibiotic treatment unless in specific conditions and clusters. HIV-positive patients are at higher risk of prolonged and severe infections from *Campylobacter jejuni* and invasive nontyphoid *Salmonellae* infections. The risk of multisite *Salmonella* infections is 300-fold increased in advanced HIV disease; hence, this condition is classified as an AIDS-defining illness.

Similarly, *E. coli*, *Shigella,* and *Clostridium* were recognized to cause more frequently diarrhea in HIV-infected patients. A large retrospective study in the United States reported *Clostridium difficile* as the most common cause of diarrhea in HIV-infected adults, accounting for more than 50% of cases [63]. In the same population *Shigella*, *Campylobacter,* and *Salmonella* were found in 14%, 13.8%, and 7.4% patients with diarrhea, respectively.

In a recent study in Kenya, typical EPEC was found in stools of HIV-infected children three times more commonly than in HIV-uninfected children, also after adjustment for confounding factors. Additionally, EPEC was identified more commonly among the immunosuppressed children [64].

Mycobacteria may cause GI infections presenting with diarrhea. Diarrhea is a relatively uncommon symptom of M. tuberculosis infection but may be more common in M. avium complex infection.

Whole stool samples and rectal swabs are both adequate for culturing typical bacteria (i.e., *Salmonella, Shigella, Campylobacter,* and *Yersinia*) and provide similar diagnostic yield. However, the diagnostic workup of HIV-infected children should include the search of Clostridium toxins and the typization of enteropathogenic *E. coli*. The use of molecular diagnostic tools may be helpful.

Bacterial diarrhea may have a severe course in HIV-infected children, and should be treated aggressively. The use of specific antibiotics should be carefully considered even in children who show a mild course of the disease, particularly in those who have a moderate-to-severe immune impairment. Some children may require empiric treatment for symptom control or prevention of the risk of extraintestinal spreading of the infection.

Viral Diarrhea

Viruses are the pathogens most frequently responsible for acute diarrhea in children, and usually have an acute and self-limiting course in immunocompetent subjects. However, viral enteropathy may be dangerous in HIV-infected children, and mainly in treatment-naive children. At least one virus was isolated in about 75% of HIV-infected children with diarrhea, compared to 65% of HIV negative, and frequently a higher viral load is detected in the stools of HIV-infected children.

Classic viral enteric pathogens include group A rotaviruses, noroviruses, astrovirus, and enteric adenovirus. More recently, emerging agents such as bocavirus and aichi virus have been considered as potential etiological agents of diarrhea. Besides, about 15% of HIV-infected children demonstrate more than one virus in the stools and without a clear relationship with intestinal symptoms.

Rotavirus is the major cause of viral diarrhea in countries where specific vaccination has not been routinely introduced. Children with HIV infection experience more severe rotavirus infections, with a higher risk of prolonged hospitalization and a higher fatality rate than non-HIV-infected children [65]. However, more recent studies in Tanzania and Brazil observed that the detection rate of rotavirus in children with acute diarrhea was higher in HIV-negative than in HIV-positive children [66].

The introduction of antirotavirus immunization is changing the epidemiology of intestinal infections in countries that promoted a large-scale implementation. However, there is evidence of a relevant variability in the immunogenicity of rotavirus vaccines across world countries. A lower immunogenicty has been observed in regions with high prevalence of HIV, such as Africa, if compared with high-income areas such as Europe of USA [67]. Although HIV infection is generally not a contraindication for immunization, high HIV prevalence in the region may result in lower rates of vaccine immunogenicity, efficacy, and population immunity.

Norovirus is fast becoming a major cause of medically attended gastroenteritis in countries with high rotavirus vaccine coverage. This agent, with a predominance of GII.4 and GII.12 genogroups, seems to be more common in HIV-infected children than HIV-negative patients and may also induce severe and chronic disease manifestations in those children [68]. A three times higher frequency of norovirus among patients with CD4+ T-lymphocyte counts <200 cells/mm^3, as well as the higher viral loads among HIV-1-seropositive children, and particularly among those more severely immunocompromised, highlights the opportunistic character of norovirus.

Comparative studies demonstrated also a higher frequency of bocavirus and adenovirus and a rare occurrence of astrovirus in seropositive children rather than healthy children.

Aichi virus, recently identified as responsible for acute diarrhea outbreaks, has been isolated in diarrheic stools of HIV-infected children with a tenfold higher frequency than those reported in HIV-negative children, suggesting the inclusion among the opportunistic pathogens infecting children with AIDS [69].

Cytomegalovirus (CMV) may act as an opportunistic enteric agent inducing severe colitis or enterocolitis, or even an intractable diarrhea syndrome in severely immunocompromised children. CMV is by far the major opportunistic etiology of colitis in HIV-infected subjects. As for other opportunistic infections, the introduction of ART significantly reduced the rate and lethality of CMV infection [70].

Clinical symptoms reported in children with CMV enteritis include chronic diarrhea, abdominal pain, and bleeding. Mucosal ulceration may sometimes result in bleeding and perforation of the large bowel. The infection of the endothelial cells and ensuing vasculitis may play a major role in the development of thrombosis, local ischemia, and ulceration of the GI mucosa. Symptomatic infection is more common and severe in children with low CD4+ counts and in those aged below 12 months. The latter may also develop a protein-losing enteropathy, with loss of albumin and immunoglobulins that further impair the child's nutritional status and immune response.

Diagnosis can be difficult because the virus may remain latent for long periods and neither serological test nor positive urine test indicates active disease. Intestinal endoscopy may show mucosal edema/erythema, hemorrhages, erosions, and ulcers; however, nearly 25% of normal gross endoscopy can have evidence of CMV infection on histopathology, with typical intranuclear and intracytoplasmic inclusion bodies [71].

In adults, CMV and other herpesviruses, as well as enteric adenovirus and bocavirus, frequently coinfect patients harboring parasites, including *Isospora belli*, *Giardia duodenalis, Strongyloides stercoralis,* and *Entamoeba histolytica/Entamoeba dispar*.

Parasitic Diarrhea

Numerous parasites cause diarrhea in HIV-infected children. The detection rate in diarrheic stools of HIV-infected children living in low-income countries varies between 24 and 57% depending on nutritional status, contact with animals, socioeconomic conditions, sanitary facilities, and access to safe water and to ART [64, 72, 73].

Either parasites with pathogenic activity in the general population (*Giardia lamblia*, *Entamoeba histolytica*, *Blastocystis hominis*, *Strongyloides stercoralis,* and other soil-transmitted helminths) or opportunistic parasites, including *Cryptosporidium parvum*, *Isospora belli, and Cyclospora cayetanensis,* may cause diarrhea in HIV-infected subjects. The spore-forming opportunistic protozoa cause intracellular infection and can lead to severe intestinal injury and prolonged diarrhea in children with impaired immunity. *Cryptosporidium* species, *E. histolytica/dispar*, *hook worm,* and *taenia* species are the intestinal parasites more frequently associated with CD4+ T-cell counts <350 cells/μμL in ART-naïve patients. The risk of opportunistic infection is almost null in children under treatment, but it is stratified according to the duration of ART (higher in the first 24–36 months) [73].

In addition to intestinal manifestation, ART-naïve children are also more prone to develop systemic symptoms related to parasite infection, such as severe anemia secondary to pathogens like hookworm*, Strogyloides stercoralis,* and *Hymenolepis nana*.

Identification of parasites may be difficult on direct microscopy and the diagnosis may be missed. The use of molecular diagnostic tools in epidemiological investigations provides new insights into species characterization and transmission pathways.

A recent study in Ethiopia used PCR-RFLP analysis of the small subunit rRNA to identify a large number of *Cryptosporidium* species and subtypes associated with different clinical manifestations in HIV-infected subjects. The two species more frequently isolated and strongly associated with diarrhea in HIV-infected children were *C. parvum* (mostly subtype IIa) and *C. hominis.* Patients with *C. hominis* or other rare species (i.e., *C. meleagridis/C. felis/C. canis,* and *C. xiaomi*) were more likely to have vomiting compared with Cryptosporidium-negative patients [74].

Differences in the geographical distribution of *C. parvum* and *C. hominis* are generally considered a reflection of differences in infection sources and transmission routes. *C. hominis* is transmitted almost exclusively among humans, whereas *C. parvum*, especially its IIa subtype family, is more likely transmitted zoonotically.

Fungal Diarrhea

Although they usually infect the upper GI tract, Candida species are frequently isolated from the stool of HIV-positive patients. Their role in determining diarrhea is still unclear since the frequency seems to be similar in HIV-infected children presenting with or without diarrhea [75]. Single reports associate Candida species infection and antibiotic-associated diarrhea [76].

Histoplasmosis that usually occurs asymptomatically in healthy children may cause disseminated infections in HIV-

infected children with variable clinical picture depending on the immunological status.

Microsporidia are obligate intracellular parasitic fungi causing chronic diarrhea, particularly among immunocompromised patients. Some species such as *Enterocytozoon bieneusi* and *Encephalocytozoon intestinalis* have been proposed as etiological agents of persistent diarrhea and failure to thrive in malnourished children. Studies in Africa (Nigeria), Asia (Malaysia), and Europe (Portugal) identified *Enterocytozoon bieneusi* as the most commonly isolated species in HIV/AIDS subjects. Children are less susceptible than adults to this kind of infection that start from the small bowel or proximal colon, causing chronic watery diarrhea and wasting, and then disseminate in most severe cases.

Noninfectious Diarrhea in HIV

Now that HIV infection is becoming a "chronic disease," at least for children and adolescents living in middle-/high-income countries with immediate and regular access to ART, other noninfectious etiologies may emerge as a differential diagnosis of chronic diarrhea, including celiac disease, inflammatory bowel diseases, or functional gastrointestinal diseases.

Usually, all HIV-infected subjects seeking care for chronic diarrhea, in the absence of an identifiable infectious cause, receive a diagnosis of "HIV enteropathy," also based on their immunological status.

There is no evidence of an increased incidence of celiac diseases in HIV-infected subjects. However, the diagnosis of celiac diseases in HIV-infected children presenting with chronic diarrhea is challenged by the possibility of false-positive tissue transglutaminase antibodies, the presence of villus atrophy on intestinal biopsy (also directly induced by HIV), and past reports of diarrhea resolution following empiric or self-administered gluten-free diets. In HIV-infected subjects, HLA typing may be essential before introducing a gluten-free diet [77–79].

Some clinical and radiological findings reported in HIV-infected subjects may mask an inflammatory bowel disease (IBD), such as severe intestinal infections with mucosal ulceration or perforation (i.e., CMV, herpes, and viruses) or the presence of bowel wall thickening (i.e., IRIS or malignancies). However, the risk of inflammatory bowel diseases seems to be similar in HIV-infected and noninfected subjects. Theoretically, HIV-related CD4+ lymphocytes depletion may be beneficial for patients with inflammatory bowel diseases. In small case series, HIV status was related to Crohn's disease remission and with a lower probability of IBD relapse [80, 81].

Pancreas and Liver in HIV-Infected Children

Exocrine pancreatic dysfunction is frequently associated with digestive dysfunction in advanced HIV. A reduction in fecal levels of pancreatic elastase and chymotrypsin has been found in up to one-third of HIV-infected children. Clinical manifestations may not be evident, but pancreatic insufficiency (fecal elastase <200 μg/g) can lead to fat and micronutrient malabsorption and should be ruled out in any patient with chronic diarrhea/steatorrhea. Selected cases may benefit from pancreatic enzyme supplementation [82, 83]. Pancreatitis may be an unusual (<2%) but serious drug adverse effect, mainly with selected nucleoside analog reverse-transcriptase inhibitors (Table 18.1) [15]. Endocrine pancreatic function is not frequent, but insulin resistance mostly secondary to dyslipidemia is seen in up to 10% children on ART and is associated with a higher body mass index but not with visible lipodystrophy [84, 85].

Liver disease is a common cause of morbidity and mortality among people living with HIV. There are several mechanisms of HIV-associated liver injury, including direct viral damage, opportunistic infections, ART-related hepatotoxicity, NAFLD, and HBV/HCV coinfection, although advancements in HIV and HCV treatment have dramatically changed the etiology and prognosis of chronic liver disease.

Liver toxicity is also a rare but potentially serious adverse event associated with ART (Table 18.1), with variable frequency according to antiretroviral regimens. Integrase inhibitors have minimal impact on liver function.

Noninvasive tools for liver function assessment are reducing the need for liver biopsy, which remains the gold standard, and are useful indicators to screen for fibrosis and cirrhosis in adults. AST-to-platelet ratio index (APRI) and fibrosis-4 (FIB-4) are, respectively, used and their role in pediatrics is promising [7, 14].

Data from a large cohort of HIV-infected children demonstrated a global incidence of liver disease at 5.8% pre-ART, and a low incidence of hypertransaminasemia post-ART initiation (less than 2/1.000 cases) in those with normal liver function at baseline. Hepatic dysfunction is more frequent in hepatitis B or C coinfected children, among whom more than half has an APRI score suggestive of fibrosis [14].

However, isolated hepatomegaly with normal or slightly raised transaminases (ALT<3 × UNL) is a relatively common finding in HIV-monoinfected children, which could be associated with nutritional deficiencies or NAFLD. Approximately 10% of children with persistent liver enzymes elevation may have underlying NAFLD and fibrosis; hence, periodic noninvasive liver assessment including liver ultrasonography and fibroscan is recommended in this subgroup [86].

Vaccination programs and newborn prophylaxis have reduced the burden of HBV in developed countries, but the global prevalence of HIV-HBV coinfection is still estimated at 1–49% according to the examined setting. Children living with HIV and HBV are at greater risk of hepatic disease and fibrosis, and the widespread use of lamivudine in ART favors the development of HBV resistance mutations. It is, therefore, essential to evaluate the HBV serostatus before the initiation of ART, and, in case of coinfection, prefer a lamivudine-sparing regimen or to include anti-HBV drugs for those who need HBV treatment [87].

HCV infection as well may be more severe in HIV-infected children than in HIV-negative individuals, as more than a quarter present with advanced fibrosis in late adolescence. Direct-acting antivirals are a safe and effective option for HCV treatment in vertically infected HIV young adults, but more data are needed for their use in adolescents and children [88].

HIV-Associated GI Malignancies

The risk of developing malignancies in HIV children is mostly related to the degree of immunosuppression and exposure to oncogenic viruses. Early ART has overall reduced the incidence of malignancies, but worldwide people living with HIV remain at higher risk. In recent estimates from a South African pediatric cohort, the most frequent cancers were the AIDS-defining Kaposi Sarcoma and non-Hodgkin lymphomas with incidence rates of 34 and 31/100,000 children per year [89].

Visceral Kaposi Sarcoma (KS) and non-Hodgkin intestinal lymphomas are HIV-associated GI malignancies and may present with nonspecific GI symptoms.

Although both are associated with the oncogenic human herpesvirus-8, HIV-associated (or epidemic) KS is a separate epidemiological entity from African (or endemic) KS, which is an aggressive tumor that affects HIV-negative children of endemic African countries and shows a predominant lymph nodal involvement.

HIV-associated KS is an AIDS-defining condition that can involve the GI tract and it is often a challenging diagnosis, especially in resource-limited settings, as it requires a high index of suspicion and availability of digestive endoscopy services. Intestinal KS may present with ascites, diarrhea, rectal bleeding, and sometimes intestinal obstruction. Upper GI KS can present with dysphagia [90].

Burkitt and Burkitt-like lymphomas and diffuse large-cell lymphomas are the subtypes that most likely affect the GI tract in HIV-infected patients and can present as bulky masses that cause intestinal obstruction [91, 92].

Nutritional Challenges in Children Growing with HIV

Many factors impact on the nutritional status of HIV-infected children, including HIV-related malabsorption, antiviral treatment, and opportunistic infections. Malnutrition enteropathy and HIV enteropathy are phenotypically indistinguishable, and they frequently overlap in clinical settings.

In HIV enteropathy, failure to thrive is usually the consequence of sugar and fat malabsorption [93]. Common micronutrient deficiencies, not exclusive of advanced disease, include iron, folate, B12, and vitamins A and E. Severe vitamin A and zinc deficiencies may increase the risk of intestinal and extraintestinal infections.

Early ART improves nutritional outcomes, although the risk of wasting in the first 5 years of life remains overall higher in HIV-infected children compared to noninfected, and poor growth correlates with delay in sexual maturation [94, 95].

Hence, a regular nutritional follow-up is recommended, particularly in resource-limited settings, and nutritional interventions may be best delivered during the first years of ART to ensure a stable growth after the catch-up phase [96]. Caloric intake should be determined quantitatively because the infection and its complications are associated with increased energy expenditure. Besides, it should be considered that several heterogeneous conditions—ranging from oral/esophageal candidiasis and ageusia, to anorexia related to ART or psychological status—can reduce food intake [12].

On the other side, the global burden of metabolic syndrome does not spare perinatally HIV-infected adolescents in both high- and low-income countries, who have higher rates of insulin resistance and dyslipidemia and may be at higher risk of pathological weight gain since the introduction of integrase inhibitors [97, 98]. These findings point toward the usefulness of an integrated nutritional and metabolic screening in HIV-infected youths—including homeostasis model assessment of insulin resistance (HOMA-IR), lipid panel, and anthropometric measurements—to detect at-risk patients who may benefit from early interventions [84, 99].

HIV and Malnutrition in Resource-Limited Settings

HIV and undernutrition are deeply connected and represent a major health challenge in most resource-limited settings. Severe acute malnutrition (SAM) is often the first AIDS-defining illness and the majority of children are not on ART at the time of admission to a nutritional rehabilitation unit.

Weight loss and SAM are also a common problem in children who are failing or nonadherent to ART. Overall, children with SAM and HIV infection have greater mortality rates (up to 30%) and more than 10% need readmission after discharge. Profuse diarrhea is one of the major drivers of morbidity and mortality in this setting [16, 100].

Currently, the nutritional management for HIV-infected children with SAM is the same as the noninfected, but there is room for the implementation of the optimal feeding regimen for such fragile patients. In the outpatient setting, the use of ready-to-use therapeutic foods for uncomplicated SAM is recognized as a useful measure to halt progression to complicated SAM [101, 102].

Vitamin A administered periodically reduces mortality. Selenium may affect positively intestinal barrier integrity and reduce systemic inflammation [103]. Iron supplementation in HIV-infected children is beneficial on immune and hematological parameters, but routine iron supplementation has not been endorsed as it increases the risk of malaria [104]. Similarly, there is discordant evidence on the use of zinc. In conclusion, despite the existing studies, there is no conclusive evidence on routine use of micronutrient supplements in nutritional programs for children living with HIV as the benefits need to be weighted in randomized controlled trials [105, 106].

Finally, several factors have to be considered when starting on ART a child with SAM, including the risk of IRIS and the high prevalence of TB in this setting.

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The Spectrum of Functional GI Disorders

19

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Introduction

Functional Gastrointestinal Disorders (FGID), also known as Disorders of Gut-Brain Interaction (DGBI), can manifest with a wide variety of symptoms caused by abnormalities within gastrointestinal motility, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota, and central nervous system gut afferent input processing. Gut dysfunction has been closely linked to emotional wellbeing and life stressors. Traditional Buddhist thinkers and Ancient Greeks Plato, Aristotle, and Hippocrates first postulated the concept that the mind and body function as singularity, referred to as holism from the Greek work *holos* or whole. In the fourth century, a paradigm shift occurred which shifted from holism to biomedicine. In 1637, Rene Descartes proposed the separation from mind and body known as dualism; this concept has dramatically affected the way we evaluate patients in modern medicine [1]. Ignorance about the biopsychosocial model of health can lead to dismissing illness without pathology as trivial or behavioral and some physicians find it difficult to empathize with their patients [2]. The concept of holism started to remerge in the nineteenth century. In 1833, William Beaumont demonstrated the association of emotions such as anger and fear with gastric mucosal morphology and function [3]. There was a surge of studies reporting the effects of emotion on gastrointestinal function (motility and sensory) in the twentieth century. These data provided evidence that the gut is physiologically responsive to external stimulation and subsequent emotional responses.

Introduction of the biopsychosocial model in the late 1970s set the stage for further research and understanding of FGID. In 1977, George Engel, an internist and psychoanalyst, challenged the tradition biomedical approach which looked at disease to be "fully accounted for by deviations from the normal of measurable biological variables" and proposed a new holistic theory that illness results in the combination of biological, psychological, and social components interacting at variable degrees [3–5]. The combination of these components determines disease severity (Fig. 19.1) [6]. This biopsychosocial model allowed better understanding of human illness by integrating biomedical thought and clinical observations, provided a framework to evaluate biomedical processes and how they are affected by psychosocial factors leading to a unique patient experience, and lastly it created a multidisciplinary team approach to assessing GI conditions by including a biopsychosocial assessment. This changed research outcomes dramatically, no longer focusing solely on morbidity and mortality but rather health-related quality of life, health care use, daily function, and symptom severity.

The complex interaction between the brain, enteric nervous system, endocrine, and the immune system, which helps to regulate the bowel function, is called the brain gut or more recently the microbiome brain-gut axis [7]. The brain-gut axis is comprised of the enteric nervous system, which is broadly organized into the myenteric and submucosal plexuses and communicates with the brain through the neural pathways, as well as the immune and endocrine systems [8]. The sympathetic and parasympathetic branches of the autonomic nervous system (ANS) connect emotional arousal and central autonomic brain circuits with the enteric nervous system. This extensive neural network innervates visceral smooth muscles and other end-organs within the GI tract and regulates the GI secretory, sensory, motor, endocrine, and immune functions. This complex network communicates information from the emotional and cognitive centers of the brain via neurotransmitters to the gastrointestinal tract and vice versa [9]. However, the bowel function can be modulated by intrinsic neural circuits within the wall of the gastrointestinal tract bypassing the central nervous system via the myenteric and

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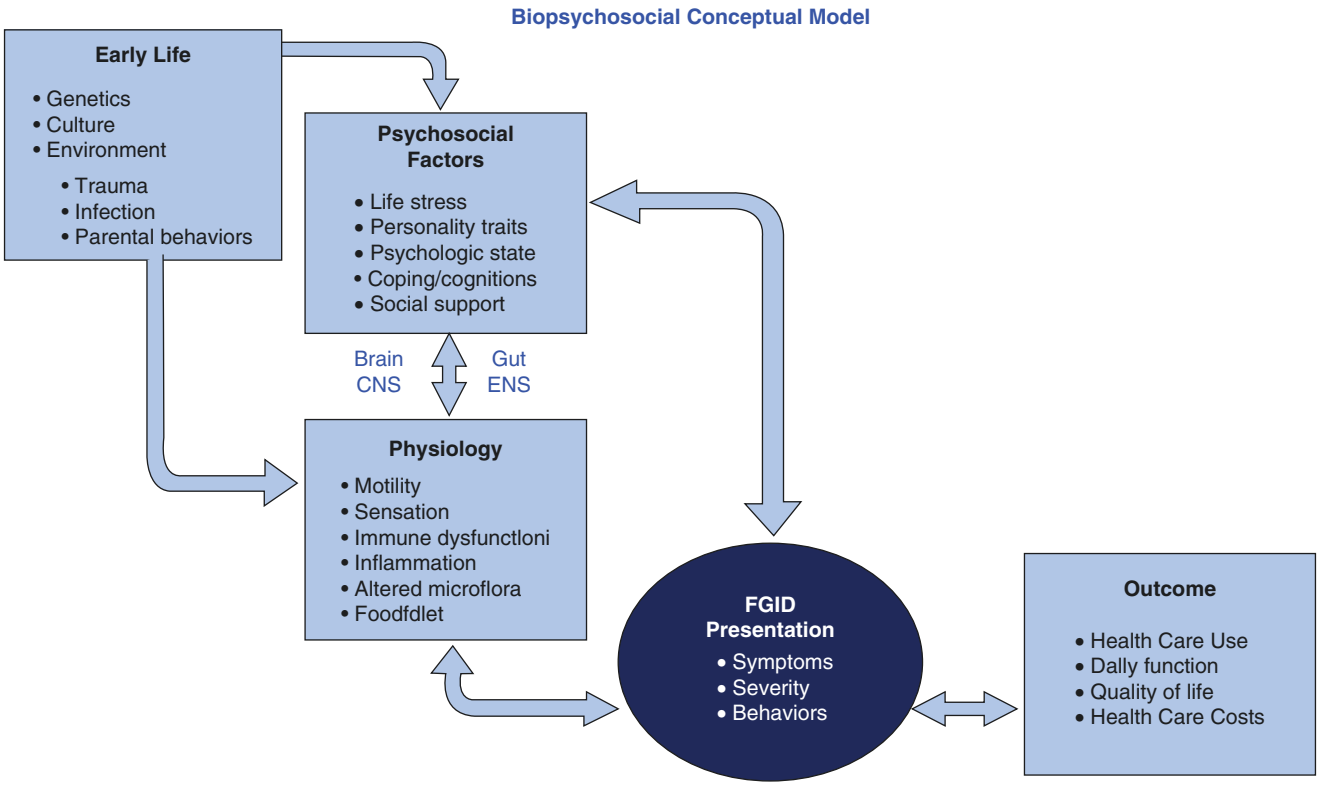

Fig. 19.1 A biopsychosocial conceptualization of pathogenesis, clinical experience, and effects of functional GI disorders. There is a relationship between early life factors (genetics, culture, and environment) that can influence the psychosocial milieu of the individual, their physiological functioning, as well as their mutual interaction (brain-gut axis). These factors influence the severity of the clinical presentation of the disorder and the clinical outcome. (Reproduced from Drossman et al. 1998 with permission from the Rome Foundation) [3]

submucosal plexuses of the enteric nervous system and other reflex circuits such as the gastrocolic and ileocolic reflexes. Gut luminal contents including the microbiome and immune system participate in this autoregulation. This complex neural network in conjunction with endocrine and immune system maintains gut and body homeostasis to ensure optimal bowel function for digestion and absorption of nutrients and elimination of waste. Symptoms can result from the disruption of gut function and homeostasis triggered by food (lactose and food intolerance), changes in gut microbiome-immune interaction (postinfectious IBS), or autonomic nervous system disease (diabetic gastroparesis) [7].

Disorders of the autonomic nervous system, such as postural orthostatic tachycardia syndrome (POTS) and diabetic neuropathy, can also manifest with gastrointestinal symptoms. These disorders are commonly associated with a broad spectrum of symptoms, for example, headaches, nausea, vomiting, and pain [10–12]. In addition to the gastrointestinal tract, the ANS also regulates cardiac function and the heart rate variability has been proposed as one of the objective measures of balance between the parasympathetic-sympathetic arms of the ANS. Gastrointestinal symptoms of autonomic dysfunction can be reproduced in the upright position with resolution of symptoms once the child is supine [13]. Frequently, patients with POTS or orthostatic intolerance may have overlapping gastrointestinal symptoms not produced by an orthostatic challenge. Orthostatic disorders are commonly associated with other comorbid conditions such as migraine headaches, fibromyalgia, chronic fatigue, nausea, sleep disorders, and abdominal pain [10]. It is important to differentiate symptoms that present with change in position versus those that are present irrespective of body position, as this will determine the treatment of choice.

Biopsychosocial Model of Functional Gastrointestinal Disorders

The main focus of the biopsychosocial model is the concept that illness is not a result of a single factor but rather a combination of factors involving early life (genetics, culture, and environment), psychosocial factors, and physiology which later impacts the interactions of the brain-gut (central nervous system-enteric nervous system) axis [7, 14, 15]. It is important that each aspect of the biopsychosocial model is addressed and treated. For example, if a patient presents with pain associated with IBS but also has depression and anxiety and a history of abuse, each of these areas can potentially

lead to the perception of pain and the patient's overall experience of illness [16]. The severity of symptoms affects the clinical outcome and vice versa. It is important to identify the severity of symptoms and limitations in daily functioning in order to determine an appropriate treatment plan. Equally important is the response from the family to the child's complaints, which may amplify the patient's symptoms and illness experience [17, 18]. When a physician validates patient's symptoms, engages them in conversation, and provides empathy, it not only builds trust but also reduces symptom severity and health care seeking behavior [19]. In the biomedical approach, both physicians and patients were in search of an organic disease as a cause for illness leading to increased health care costs due to referrals, tests, medications, and possible surgeries. This can lead to increased anxiety, frustration, and dissatisfaction among both the physician and patient. Breaking this cycle and approaching patients with DGBI by a biopsychosocial model is imperative to improving their quality of life and health care outcomes.

Genetic susceptibility influences future behaviors and experience of illness. DGBI can result from polymorphisms affecting the motor function, membrane permeability, and visceral sensitivity, which has been reported in patients with IBS [20, 21]. In addition, stress may affect epigenetic expression of these genes leading to visceral hypersensitivity and other motor function abnormalities in DGBI [22].

Culture plays an important role in the child's expectation and perception of their illness. Fitting in with the cultural and society norms and functioning as a meaningful contributor to society is critical to psychological wellbeing of an individual. Based on cultural norms and influences, this determines whether a patient seeks medical attention, is self-treated, or ignored. For example, in Mexico diarrhea is often not seen as an illness requiring medical attention since it is so common [23]. In Arapesh, pregnant women did not report morning sickness because they did not believe the child existed until after birth [24]. Even expression of pain varies among cultures [25]. The role of a physician is also very important within cultures, where some cultures accept patient-centered care as a norm with shared decision-making, where other cultures see this as a sign of weakness or lack of knowledge on behalf of the physician [26]. It is critical to understand the patient's belief system and set of cultural norms in order to involve them in decision-making.

Early life events involving feeding and elimination are often the first experiences in a child's life of confrontation. According to the psychoanalytic theory, the child's early innate impulses to eat and defecate meet external confrontation and naturally, they are prone to resolution of these conflicts. With time, they will learn to comply or resist environmental control of these functions by refusing to eat, defecate, or acting out. The behaviors learned during this time frame are pivotal in the development of autonomy, learning right from wrong, and adopting socially acceptable behaviors such as bowel functioning. Functional defecation disorders, for example, infant dyschezia and learned feeding disorders can develop due to confrontation experienced during early life [27]. Other children will develop abnormal patterns of defecation out of defiance or control leading to fecal incontinence and pain as toddlers.

Unresolved stress due to traumatic life events or daily life stressors can impact the way an individual experiences their illness in various ways (1) producing psychophysiological effects on gastrointestinal motor and sensory functioning, (2) amplifying symptoms due to brain processing of afferent gut input such as hypervigilance, and (3) developing poor coping skills and health care seeking [28–30]. It is important to recognize chronic and daily life stressors when establishing a treatment plan, as these events can lead to poor outcomes and decreased quality of life. A history of physical or sexual abuse has been linked to symptom severity and outcomes. Inflexibility and inability to recover or adapt to adverse early life events are possible mechanisms for increased risk of DGBI [31]. In cases where individuals have developed maladaptive behaviors, behavioral intervention is needed to correct these behaviors and change the way individuals view their illness [29].

Parental beliefs and behaviors through a child's life can have a positive or negative impact on how a child experiences illness. A child of a mother who reinforces illness behavior will have more reported severe abdominal pain and school absences than a child whose mother does not reinforce this behavior [17]. In a recent clinical trial, children reported more pain when parents showed sympathetic response to their complaints, compared to parents who ignored the complaints [32]. In addition, children's abdominal pain has been shown to be associated with parental anxiety and depression [33]. A child learns how to cope with various situations in life based on modeling their parent's response to their own experiences, the same way that parent's personality traits may also influence illness experience. For example, a parent who excessively worries or catastrophizes may also reward a child's somatic complaints reinforcing this illness behavior.

A child's psychological status may influence gastrointestinal physiology, leading to the development of a DGBI and its symptomatic and behavioral expression influencing outcomes. Psychological factors can be divided into either longstanding, or trait, features such as personality or psychiatric disorders and short-term, or state, features such as psychological distress. Though a patient's symptoms of anxiety and depression may not meet the criteria based on current psychiatric classification systems, this does not discredit their potential impact on functional gastrointestinal symptoms. Comorbid depression has been linked to poor outcomes in patients with DGBI, including increased health

care utilization, decreased functioning, poor treatment compliance, and overall poor quality of life [29]. Anxiety leads to increased autonomic arousal, which can interfere with gastrointestinal motility and sensitivity leading to hypervigilance and decreased pain tolerance [34]. Recognizing these conditions and familiarizing oneself with psychological and psychopharmacologic interventions can affect the long-term outcome of patients with DGBI [35, 36].

Approach to Patients with Disorders of Gut-Brain Interaction

A careful consideration of the biopsychosocial model in approaching patients with DGBI is critical in patient satisfaction and outcomes. Effective communication is an essential part of developing a trusting patient-physician relationship. Overreliance on technology to the detriment of effective communication can be counterproductive. Focusing on the four main principles of effective communication (active listening, addressing the patients' agenda, providing empathy, and validation of patients' beliefs and concerns) aids in improving diagnosis and clinical decision-making by creating a trusting environment in which patients feel comfortable sharing both their clinical and psychosocial information [37, 38]. This creates a holistic view of the patients' symptoms, allowing a provider to see the full impact it has on their health care quality of life. Effective communication allows patients to collaborate in their treatment plan through shared decision-making, improving patient compliance, and motivating them to share the responsibility of their disease burden. Effective verbal and nonverbal communication can decrease overall time spent on making a positive diagnosis by forming a trusting relationship where the patient feels comfortable sharing personal information and participating in shared decision-making. It allows the patient to feel heard, sharing their expectations and goals for the encounter, allowing for improved outcomes for the patient by reducing symptom severity, emotional distress, improves satisfaction and coping, improves adherence to treatment and decreased overall health care costs. For the provider, effective communication skills training has been shown to decrease emotional exhaustion and burnout. The provider-patient relationship is the most commonly reported indicator for physician satisfaction [39]. The Rome Foundation has made efforts to improve education for medical trainees by offering free study guides to improve communication skills (https://romedross.video/2YphMDd) and for self-learning educational videos (https://romedross.video/2KPTYzC). Table 19.1 is a list of verbal and nonverbal methods that can be applied to improve patient-provider communication [37]. In order to

Table 19.1 Verbal and nonverbal behaviors affecting communication

Behavior	Facilitates	Inhibits
Nonverbal		
Clinical environment	Private, comfortable	Noisy, physical barriers
Eye contact	Frequent	Infrequent or constant
Listening	Active listening—questions relate to what the patient says	Distracted or preoccupied (e.g., typing)
Body posture	Direct, open, relaxed	Body turned, arms folded
Head nodding	Well time	Infrequent, excessive
Body proximity	Close enough to touch	Too close or too distant
Facial expression	Shows interest and understanding	Preoccupation, boredom, disapproval
Voice	Gentle tone	Harsh, rushed
Touching	Helpful if well timed and used to communicate empathy	Insincere in inappropriate or not properly timed
Synchrony (arms, legs)	Concordant	Discordant
Verbal		
Question forms	Open ended to generate hypothesis	Rigid or stereotyped
	Closed ended to test hypothesis	Multiple choice or leading questions ("You didn't… did you?"
	Use of patient's words	Use of unfamiliar words or jargon
	Facilitates patient discussion by "echoing" or affirmative gestures	Interruptions, undue control of conversation
	Uses summarizing statements	Not done
Question/Interview style	Nonjudgmental	Judgmental
	Follows the lead of patient's prior comments (patient centered)	Follows own preset agenda or style
	Use of narrative thread	Unorganized questioning
	Appropriate use of silence	Interruptions or too much silence
	Appropriate reassurance and encouragement	Premature or unwarranted reassurance or encouragement
	Communicated empathy	Not provided or not sincere
Recommendations	Elicits feedback and negotiates	No feedback, directly states views
Asks/provides medical information	As appropriate to the clinical issues	Too many biomedical questions and too detailed information

(continued)

Table 19.1 (continued)

Behavior	Facilitates	Inhibits
Asks/provides psychosocial information	Elicits in a sensitive and nonthreatening manner	Ignores psychosocial data or asks intrusive or probing questions
Humor	When appropriate and facilitative	None or inappropriate humor

Permission from the Rome Foundation [37]

Table 19.2 Rome IV Foundation 12-step approach to patients with DGBI

1. Improve patient satisfaction and engage the patient in the visit through verbal and nonverbal communication
2. Obtain a history through active listening and a non-judgmental, patient centered interview
3. Establish patient expectations and reason for the visit
4. Well-administered physical exam and directed investigations
5. Determine what the patients understands as the underlying cause of their condition and concerns regarding outcomes
6. Identify patient understanding of their symptoms and provide an explanation taking the patients beliefs into consideration
7. Reconcile patients' expectations on improvement and the provider's ability to help
8. Explain how stressors can impact symptoms consistent with patients' belief system
9. Set boundaries
10. Shared decision-making
11. Make treatment recommendations consistent with patient interests and beliefs
12. Establish long-term relationship with patient and primary care provider

Adapted from Drossman et al. 2016 [3]

improve patient satisfaction, adherence to treatment, and outcomes, the Rome IV Foundation created a 12-step approach to building patient-physician relationships with patients with DGBI (Table 19.2) [3].

Physical Exam

The physical exam is a rite of passage for medical professionals. The significance of a physical exam is more profound than simply the structure of the human body [40]. The human body embodies not only bones and organs but is a symbol of history, culture, and politics. The concept of embodiment rejects mind/body dualism and looks at the body as a whole situated in society to better understand how illness and pain further defines how an individual lives their human experience [40, 41]. The role of the physician in a white coat and the patient donning a gown signifies a power imbalance, allowing the physician to lie his/her hands on the body implies vulnerability and trust on behalf of the patient. These actions must be done attentively and compassionately to preserve the trust and respect of the patient and further deepen the patient-provider relationship.

The role of the physical exam is not only to diagnose a specific problem but may have a positive or negative impact on the patient depending on how it is performed. Studies completed on the placebo effect have demonstrated that it is not only the pill that is responsible for the neurobiological effects on the patient but the ritual surrounding the patient-provider encounters including positive expectations [42]. On the other hand, there can be a nocebo effect, where negative expectations may have an adverse effect on the patient [43]. Taking into consideration the placebo and nocebo effects, a physical exam administered with warmth and empathy can have a positive impact on the patient and aid in building a stronger patient-provider relationship. This can also lead to increased satisfaction and meaningfulness in the provider's practice.

Symptom-Based Approach to Functional Gastrointestinal Disorders

Over the past 30 years, the Rome Foundation has forged a path for research on FGID and developed symptom-based criteria to diagnose FGID. The Foundation has emphasized that in order to advance the field of FGID, we need to address the following: (1) the term "functional gastrointestinal disorders" lacked precision and carries some degree of stigma. (2) The diagnostic criteria are not practical in the clinical setting and lack meaningful subsets of diagnoses to identify physiological biomarkers that may lead to targeted treatment options. (3) The degree to which psychological comorbidities impact the severity of the condition, degree of disability, and centrally mediated treatment options was unclear. (4) Lack of investigative pathways to determine proper diagnostic testing based on severity and disability prior to implementing the diagnostic criteria. (5) Lack of cultural diversity in knowledge acquisition. (6) Multiple changes were made to the Rome IV diagnostic criteria released in 2016 to address previously recognized limitations including removing the term "functional" when not needed to improve specificity and decrease potential stigma associated with these conditions.

The current Rome IV diagnostic criteria are based on symptoms rather than physiological criteria, which makes them more practical for clinical use. The disorders are classified into anatomic regions, with pediatric DGBI further categorized into neonate/toddler and child/adolescent DGBI (Table 19.3) [6]. The presentation of DGBI is dependent on the age and stage of development. Functional symptoms of childhood may accompany normal development or may arise from abnormal internal or external stimuli such as the retention of feces in the rectum due to a history of painful bowel movements, which leads to maladaptive behavioral responses.

One of the limitations of using symptom-based diagnostic criteria in pediatrics is the difficulty in getting an accurate

Table 19.3 Functional GI disorders: Neonate/toddler and child/adolescent

Functional nausea and vomiting disorders
Infant regurgitation[a]
Cyclic vomiting syndrome (CVS)
Functional nausea and functional vomiting
Functional nausea
Functional vomiting
Rumination syndrome
Aerophagia
Functional abdominal pain disorders
Infant Colic[a]
Functional Dyspepsia
Postprandial distress syndrome
Epigastric pain syndrome
Irritable bowel syndrome (IBS)
IBS with predominant constipation
IBS with predominant diarrhea
IBS with mixed bowel habits
IBS unclassified
Abdominal migraine
Functional abdominal pain – NOS
Functional defecation disorders
Functional diarrhea[a]
Infant dyschezia[a]
Functional constipation
Non-retentive fecal incontinence

Adapted from Drossman et al. 2016 [6]
[a]Conditions found only in neonates and toddlers

description of the symptoms and associated triggers, especially in young children. Further, the Rome criteria alone do not address the psychological impact on illness behavior, functional disability, and severity of disease, which influence treatment and outcomes.

Prevalence

Functional gastrointestinal disorders are common disorders among children and adolescents. In a large-scale prevalence study of US children ages 4–18 years, 23.1% of the children qualified for at least one DGBI. The most common DGBI were functional constipation and abdominal migraine. Children who met the criteria for DGBI have lower quality-of-life scores than those without a DGBI. Children were also more likely to have a DGBI, if the parent also had a DGBI[44].

Functional Nausea and Vomiting Disorders

There are a spectrum of disorders that fall under this category, including cyclic vomiting syndrome (CVS) and abdominal migraines (see Chap. 28). In chronic functional nausea, the bothersome symptom is nausea, which occurs at least twice weekly for a minimum of 2 months. The symptom is generally not associated with meals or vomiting. Functional nausea can occur in conjunction with other pain-predominant DGBI. Recent data indicate a high number of comorbidities and psychosocial disability [45]. Autonomic disorders, such as POTS, are frequently associated with refractory nausea, particularly in adolescent females [10]. Family history of migraine is commonly reported. Based on clinical presentation, predominant symptoms, and severity of disability, a clinician can determine the appropriate diagnostic and treatment plan. Studies have shown that extensive diagnostic workup has a low yield in the absence of red flags [46]. With a detailed history and physical exam, diagnosis may be made prior to the recommended time frame in the Rome criteria. It is important to consider additional etiologies, which may mimic these conditions such as intestinal malrotation and ureteropelvic junction obstruction [47–50]. Treatment is phenotype specific. Although pediatric data are sparse, empiric therapy with tricyclic antidepressants and cyproheptadine at similar doses as used for CVS prophylaxis is generally first-line therapy (see Chap. 28). Other migraine agents and anticonvulsants, such as topiramate or valproic acid, can be considered, especially in refractory cases [51].

Functional Abdominal Pain Disorders

Functional abdominal pain disorders (FAPD) can be subclassified into functional dyspepsia, irritable bowel syndrome (IBS), abdominal migraine, and functional abdominal pain – not otherwise specified (FAP-NOS) depending on specific details regarding pain location, severity, quality, and associated symptoms (Table 19.3). It is important to identify any red flags such as unintentional weight loss, blood in the stools, persistent right upper or lower abdominal pain, persistent vomiting, dysphagia, odynophagia, arthritis, family history of inflammatory bowel disease, or nocturnal diarrhea which may prompt additional work or endoscopy evaluation.

Since varied factors can contribute to the development and progression of FAPD, the management of these disorders can often involve multiple treatments and should be tailored for individual patient needs based on the severity and duration of their symptoms. Management begins with a thoughtful discussion of the diagnosis and treatment options with the family and the child. The biopsychosocial model of FAPD development and tailoring treatment strategies to the individual child are important. Dietary triggers should be identified and eliminated, and cognitive behavioral therapy (CBT) has been shown to be very effective [52]. Four types of psychotherapies have been identified to be the most beneficial for patients with DGBI: cognitive behavioral therapy, psychodynamic interpersonal therapy, mindfulness/acceptance-based therapy, and gut-directed hypnotherapy [53–55].

Mild Symptoms

Patients with minor symptoms, which are infrequent, not affecting daily activities, causing psychological distress, or leading to increased visits to the doctor's office, can be managed by education, reassurance, and diet. They tend to have less psychiatric comorbidities and a better quality of life. Supplements such as peppermint oil or herbal combination preparation STW 5 (Iberogast®) have been shown to improve abdominal pain in patients with IBS and functional dyspepsia [56, 57]. A food diary may be helpful for patients to identify certain triggers leading to worse symptoms.

Moderate Symptoms

Patients who have intermittent disruptions in their daily lives, missing occasional school days, other activities, and a more frequent symptom profile with poorer quality of life require closer follow-up of symptoms and monitoring of psychological stressors. These patients would benefit from keeping a symptom diary for 1–2 weeks and associated triggers. Pharmacotherapy should be directed at the predominant symptom causing the most disruption in daily life. Asking the patient, "Of all the symptoms we discussed today, which one is the most bothersome?" is very helpful in improving patient satisfaction and outcomes. Psychological treatment should be utilized in patients who identify specific stress triggers and who are motivated to participate. Multiple therapies have been shown to decrease anxiety and improve quality-of-life measures such as cognitive behavioral therapy, mindfulness, meditation, and relaxation [34].

Severe Symptoms

Patients with severe symptoms of DGBI are best cared for by a multidisciplinary team involving psychiatry, psychology, nutrition, and gastroenterology and pain management. In addition to the gastrointestinal symptoms, these patients have significant psychological comorbidities and dysfunction in daily life and poor quality of life. They have increased psychological distress due to chronicity of their symptoms and often have comorbid psychiatric conditions such as depression and anxiety. These patients may suffer from early childhood trauma, decreased coping skills, and a poor social support system. In their previous health care experience, they may have felt stigmatized with their condition being told, "It's all in their head" and deny any potential involvement of psychosocial factors often refusing psychological and psychiatric treatments. They often seek multiple opinions with unrealistic expectations of a cure for their symptoms. Establishing a trusting patient-provider relationship is the foundation for treatment of these patients and can prevent "doctor shopping" behavior. Patients must understand that the provider is listening to their concerns and is addressing their complaints without bias. The Rome IV 12-step approach to patients withDGBI addresses the main principles in establishing a solid patient-provider relationship while improving patient satisfaction and outcomes. In addition to the 12- step approach, providers should perform diagnostic and therapeutic interventions based on objective findings rather than at the request of the patient. Predominant psychiatric comorbidities (anxiety and depression) in need of treatment should be identified and treated appropriately. The Rome IV Foundation working team published a guide on Neuromodulators for Functional Gastrointestinal Disorders (Disorders of Gut-Brain Interaction) for treatment of psychiatric comorbidities and chronic pain syndromes. See Fig. 19.2 for pharmacological treatment options [36].

Gastrointestinal tract activity is mediated through neurotransmitters and neuropeptides, which are found in both the central nervous system and the intestine. These substances can impact both human behavior and GI function. It is important to identify which areas of the brain-gut access are most affected to guide the treatment plan. As pain becomes more severe, patients may develop additional comorbidities as they suffer from chronic pain leading to the need for additional psychological and behavioral interventions. Acetylcholine is the primary excitatory neurotransmitter in the parasympathetic nervous system that drives motility in the gastrointestinal tract. Disturbances in acetylcholine secretion and metabolism can have a major impact on motility and secretion in the gut leading to gastroparesis and constipation. Sympathetic division of the autonomic nervous system participates in neuromodulation via serotonin, norepinephrine, and dopamine. They act primarily by inhibiting activity in the gastrointestinal tract by decreasing secretions, motility, and sphincter relaxation. Modulation of the serotoninergic system has been shown to affect the pain threshold in patients with DGBI.

Functional Defecation Disorders

Functional defecation disorders are the most commonly reported DGBI. Functional constipation can present as early at the neonatal period (see Chap. 27). Recognizing this condition and treating appropriately has major health care implications. In a Dutch tertiary hospital, one-fourth of children diagnosed with functional constipation continued to experience symptoms into adulthood. Risk factors for poor clinical outcomes in adulthood were identified, including late referral to a specialized clinic after failing first-line therapies [58]. Consider other conditions that may mimic functional constipation such as ultrashort segment Hirschprung disease and

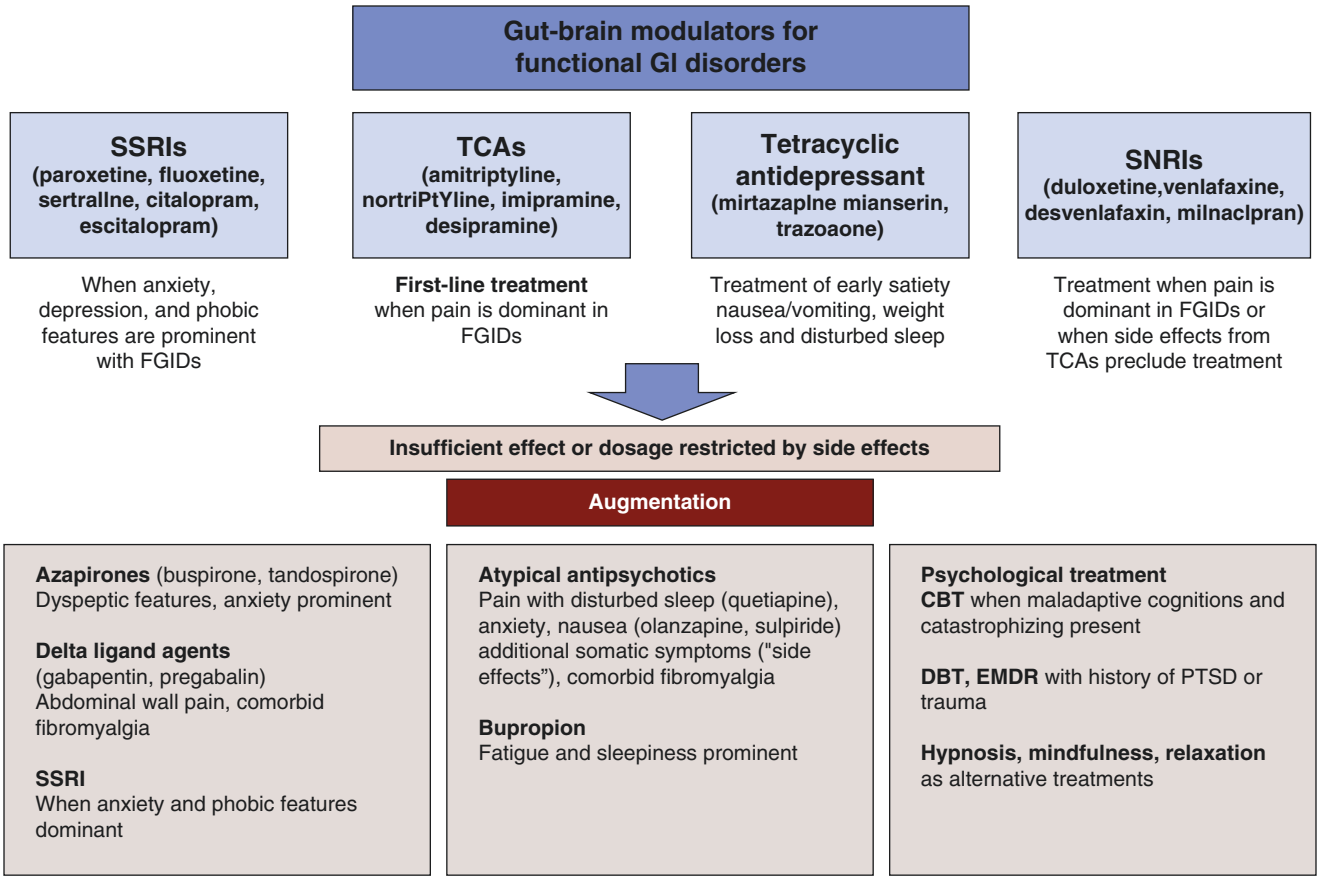

Fig. 19.2 Summary of the clinical characteristics that can be considered when selecting gut-brain neuromodulating pharmacotherapy to treat FGID. Those drugs in the upper part of the figure can be considered as first-line options. In the lower part of the figure, the pharmacologic options most often used to augment treatment effects are depicted, as well as some nonpharmacologic treatment alternatives. (DBT—dialectical behavior therapy, CBT—cognitive behavioral therapy, EMDR—eye movement desensitization and reprocessing) (Reproduced from Drossman et al. 2018 with permission from the Rome Foundation) [36]

anal stenosis in a child who presents in infancy with constipation. In addition, it is necessary to consider inflammatory conditions in toddlers who present with nonretentive fecal incontinence.

In constipation predominant irritable bowel syndrome, the lower abdominal pain persists, despite adequate laxative therapy. Soluble fiber such as methylcellulose and adequate fluid intake are generally recommended. Tricyclic antidepressants can worsen the constipation because of the anticholinergic activity, and agents with less anticholinergic activity are generally preferred for pain modulation [36].

Infant Dyschezia

Uncoordinated defecation can result in discomfort, crying, and straining in an infant. It is associated with the passage of soft stool several times a day. The disorder can be confused with functional constipation or infantile colic. The reported prevalence of dyschezia is between 0.9% and 5.6% and most infants improve by 3–6 months of age. Management involves parental reassurance and support. Overuse of laxatives or stimulants should be avoided, as they are generally not helpful. Instead, teaching the parents to flex the hips on the abdomen to relax the pelvic floor, gentle massage of the abdomen and of the perineum may help the infant to have a successful bowel movement.

Functional Diarrhea

This is characterized by passage of four or more unformed stools for ≥4 weeks with onset in infancy or preschool years in an otherwise healthy and thriving child. It is also known as Toddler diarrhea or chronic nonspecific diarrhea. Parents often report stool containing undigested food, especially vegetables like peas, carrots and corn. Rapid oro-anal transit due to immature bowel motility and excessive dietary intake of fructose, for example, fruit juices or squash have been implicated in the pathophysiology. It is important to differentiate this from malabsorption disorders and celiac disease (see Chaps. 38, 39, and 41).

Disorders of gut-brain interaction are a complex group of disorders that require a holistic approach to patient care. By taking time to listen to each patient and their story, the provider can create and long-lasting patient-provider relationship that not only improves patient outcomes but also provides meaning and satisfaction to the practice of medicine.

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Disorders of Sucking and Swallowing

20

Francesca Paola Giugliano, Erasmo Miele, and Annamaria Staiano

Introduction

The development of feeding skills is an extremely complex process influenced by multiple anatomic, neurophysiologic, environmental, social, and cultural factors. Oral feeding in infants should be efficient to preserve energy for growing. Moreover, it should be safe so as to avoid aspiration, and it should not compromise respiratory status. This can only be achieved if sucking, swallowing, and breathing are properly coordinated [1]. This entire process is dynamic because of ongoing growth and development. Functional feeding skills, which depend on the integrity of anatomic structures, undergo changes based on neurologic maturation and experimental learning. Eating/feeding requires active effort by infants who must have exquisite timing and coordination of sucking, swallowing, and breathing to be efficient [2].

A variety of neurological, neuromuscular conditions in children and infants can impair the physiological phases of sucking/swallowing and cause disorders of feeding and dysphagia. The causes of feeding and swallowing problems include combinations of structural deficits, neurologic conditions, respiratory compromise, feeder–child interaction dysfunction, and numerous medical conditions, such as genetic, metabolic, and degenerative disease [3].

In recent years, there has been an increase in infant swallowing disorders as a result of improved survival rates for infants born prematurely or with life-threatening medical disorders. Negative experiences related to feeding, such as intubation, tube feeding, or airway suctioning may further disturb sucking and swallowing development [4]. Disorders of feeding and swallowing in children are serious and potentially fatal problems. Aspiration due to dysphagia may lead to severe pulmonary disease, and impaired oral and pharyngeal function may rapidly result in failure to thrive. Prompt evaluation of swallowing disorders is therefore critical.

The differential diagnosis of dysphagia in children is wide. The diagnostic workup can be extremely difficult and exhaustive in many cases. Because of this complexity, multidisciplinary team evaluations should be conducted.

Successful rehabilitation of children with swallowing disorders requires knowledge of the parameters of normal and abnormal swallowing plus skill in the integration of a variety of essential therapeutics techniques.

Epidemiology

Data on the incidence of swallowing disorders are lacking, because in clinical practice, disorders of swallowing are often considered in the general context of a feeding disorder. Feeding (or eating) is different from swallowing. Eating is primarily an oral phase function that includes oral preparation and oral transit of a bolus [5]. Feeding is a complex process that involves a number of phases in addition to the act of swallowing, including the recognition of hunger (appetite), the acquisition of the food, and the ability to bring the food to the mouth [6]. The estimated prevalence of feeding problems in the pediatric population ranges from 25% to 35% in normally developing children to 40–93% in children with developmental delay [7, 8]. Early sucking and swallowing problems were reported to be present in 35–48% of infants with different types of neonatal brain injury [9]. However, knowledge of the true epidemiology of pediatric dysphagia remains largely unavailable because of the lack of a standardized reporting system assessing dysphagia in all of the possible contexts that may occur in infants and children [10].

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Etiology

Disorders of sucking/swallowing may be caused by multiple etiologic factors that may interfere with the child's ability to coordinate swallowing and breathing maneuvers and may be manifested as a unique set of symptoms. Potential causes responsible for three broad categories include (1) immaturity, delay, or a defect in neuromuscular control; (2) anatomic abnormality of the aerodigestive tract; (3) systemic illness. The magnitude of the dysfunction depends on the balance between the extent of the structural or functional abnormality and the child's compensatory adaptations [11]. Disorders associated with sucking/swallowing difficulties are reported in Table 20.1 [12, 13].

Pathophysiology

The fetus is capable of swallowing amniotic fluid in utero, indicating that the motor program for swallowing is functioning before gestation is complete.

However, oral feeding is not initiated in preterm infants before 32 weeks of postconceptional age, partly because the coordination of sucking, swallowing, and respiration is not established [14]. Even at 34 weeks, minute ventilation during sucking decreases more than that of infants at 36–38 weeks. Therefore, the coordination between swallowing and breathing is not yet fully organized at 34 weeks of postconceptional age [15, 16].

Anatomic structures, which are essential to competent feeding skills, undergo growth that changes their physical

Table 20.1 Differential diagnosis of dysphagia

Prematurity		
Upper airway obstruction	Nasal and nasopharyngeal	Cohanal atresia, stenosis, septal deflections and abscess, infections, tumors, sinusitis
	Oropharynx	Defects of lips and alveolar processes, cleft lips or palate, hypopharyngeal stenosis, craniofacial syndromes or sequences (e.g., Cruzon, Treacher Collins syndrome, and Pierre Robin sequence)
	Laryngeal	Laryngeal cleft and cyst, laryngomalacia, subglottic stenosis, and paralysis
Congenital defects of the larynx, trachea, and esophagus	Laryngotracheoesophageal cleft	–
	Tracheoesophageal fistula with associated esophageal atresia	–
	Esophageal anomalies (e.g., strictures, webs)	–
	Vascular anomalies	Aberrant right subclavian artery
		Double aortic arch
		Right aortic arch with left ligamentum
Acquired anatomic defects	Trauma	External trauma, intubation, endoscopic, and foreign body
	Chemical ingestion	–
Neurologic disorders	Central nervous system	Trauma
		Hypoxia and anoxia
		Cortical atrophy, hypoplasia, agenesis
		Infections (meningitis, brain abscess)
Peripheral nervous system disease	Trauma	–
	Congenital defects	
Neuromuscular	Guillan-Barre syndrome	
	Poliomyelitis (bulbar paralysis)	
	Myasthenia gravis	
	Myotonic muscular dystrophy	
Anatomic and functional defects	Cricopharyngeal dysfunction	
	Esophageal achalasia	
	Esophageal spasm	
	Paralysis of the esophagus	
	Associated atresia-tracheoesophageal fistula, nerve defect	
	Peptic and eosinophilic esophagitis	
	Riley-Day syndrome (dysautonomia)	
	Brain stem compression (e.g., Chiari malformation, tumor)	

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relationship to one other and consequently affects their function. The swallowing mechanism, by which food is transmitted to the stomach and digestive organs, is a complex action involving 26 muscles and 5 cranial nerves. The neurophysiologic control involves sensory afferent nerve fibers, motor efferent fibers, paired brainstem swallowing centers, and suprabulbar neural input. Structural integrity is essential to the development of normal feeding and swallowing skills [17]. The central patterning of aeroingestive behavior is based on volitional and reflexive control mechanisms and benefit from sensory feedback to modify the spatiotemporal organization of the feed sequence to allow safe swallow [18]. Central pattern generators (CPGs) are primarily composed of adaptive networks of interneurons that activate groups of motor neurons to generate task-specific motor patterns [19]. The essential components of the masticatory CPG are found between the rostral poles of the fifth and seventh motor nuclei. Although they are normally synchronized by commissural axons, each hemisection side can generate a rhythm [20]. Mastication patterns differ greatly between foods and change systematically during a chewing sequence based on sensory feedback. Functional imaging has revealed that swallowing is controlled through a network of cortical areas which shares loci with other ororhythmic movements including speech [21].

Deglutition is generally divided into phases of swallowing based on anatomic and functional characteristics: preoral, pharyngeal, and esophageal [22, 23].

Anatomic Considerations

An understanding of the anatomy of the pharynx is essential to a thorough understanding of the swallowing process. The anatomy changes during development. The tongue, the soft palate, and the arytenoids mass (arytenoids cartilage, false vocal cords, and true vocal cords) are larger relative to their surrounding chambers when compared with the adult [24]. In the infant, the tongue lies entirely within the oral cavity, resulting in a small oropharynx [25, 26]. In addition, a sucking pad, composed of densely compacted fatty tissue that further reduces the size of the oral cavity, stabilizes the lateral walls of the oral cavity. The larynx lies high in the infant, and the tip of epiglottis may overlap the soft palate. These anatomic relationships are ideal for the normal infant feeding pattern of suck or suckle feeding from a breast or a bottle in a recumbent position [26]. In the infant, the larynx sits high in the neck at the level of vertebrae C1 to C3, allowing for the velum, tongue, and epiglottis to approximate, thereby functionally separating the respiratory and digestive tracts. This separation allows the infant to breathe and feed safely. By age 2–3 years, the larynx descends, decreasing the separation of the swallowing and digestive tracts [7].

Development and Normal Swallowing Function

Swallowing skills develop progressively during fetal and neonatal maturation [27]. At approximately 26 days' fetal age, the developmental trajectories of the respiratory and swallowing systems diverge and start to develop independently. Swallowing in fetuses has been described as early as 12–14 weeks' gestational age. A sucking response can be provoked at 13 weeks' postconceptional age by touching the lips [28]. Real sucking, defined by a posterior–anterior movement of the tongue, in which the posterior movement is dominant, begins at 18–24 weeks' postconceptional age [29]. Between 26 and 29 weeks' there is probably no significant further maturation of sucking [30]. By week 34, most healthy fetuses can suck and swallow well enough to sustain nutritional needs via the oral route, if born at this early age. Sucking movements increase in frequency during the final weeks of fetal life due to an increase in amniotic fluid swallowed by a fetus during pregnancy from initially 2–7 ml a day to 450 ml a day. This is approximately half of the total volume of amniotic fluid at term [31]. The normal maturation of sucking and swallowing during the first months of life after full-term birth can be summarized by increased sucking and swallowing rates, longer sucking bursts, and larger volume per suck [16]. The skill of safe and efficient oral feeding is based on oral-motor competence, neurobehavioral organization, and gastrointestinal maturity [32]. Two forms of sucking are distinguished: nutritive sucking (NS) and non-nutritive sucking (NNS). NS is an infant's primary means of receiving nutrition while NNS is regarded as an initial method for exploring the environment. The rate of NNS is approximately twice as fast as that of NS [33]. In NS, however, the ability to integrate breathing with sucking and swallowing is essential for coordinated feeding [1] and it becomes consistent by 37 weeks' gestation [34]. By increasing the intra-oral space, the infant begins to suppress reflexive suckle patterns and starts to use voluntary suck patterns. In contrast to suckling, true sucking involves a raising and lowering of the body of the tongue with increased use of intrinsic musculature. Most of the infants complete the gradual transition from suckling to true suck by 9 months of age. This is considered a critical step in the development of oral skills that will permit handling of thicker textures and spoon-feeding [35].

As with sucking, chewing patterns emerge gradually during infancy. Between birth and 5 months of age, a phasic bite-release pattern develops. At this series of jaw openings and closing begins as a reflex and evolves into volitionally

controlled bite. True chewing develops as the coordinated activity of the tongue, cheeks, and jaws to participate in the breakdown of solid food. The eruption of the deciduous teeth between ages of 6 and 24 months provides a chewing surface and increases sensory input to facilitate the development of chewing [35].

The concept of a "critical period" is relevant to feeding development. A critical period refers to a fairly well-delineated period of the time during which a specific stimulus must be applied to produce a particular action. After such a critical period, a particular behavior pattern can no longer be learned. Critical periods have been described for chewing and for taste. The critical period for chewing is that time following the disappearance of the tongue protrusion reflex that should occur around 6 months of age [36]. Critical periods have also been reported for introduction of tastes. Newborn infants detect sweet solutions, reject sour flavors, and are indifferent to the taste of salt [37]. Mc Farland and Tremblay emphasized that sensory experience is crucial to optimize pattern formation and brain development during the critical period for attainment of swallowing proficiency [38].

Current knowledge of the swallowing mechanism is derived mainly from radiographic studies, which have been in use since the early 1900s. Plain films of the pharynx were replaced in the 1930s by cineradiography, which was subsequently in the 1970s replaced by videofluoroscopy. Videofluoroscopy permits instant analysis of bolus transport, aspiration, and pharyngeal function [39]. Using this descriptive method, deglutition can be divided into four phases: oral preparatory phase, oral voluntary phase, pharyngeal phase, and esophageal phase (Table 20.2) [13, 40].

The oral preparatory phase occurs after food is placed into the mouth. The food is prepared for pharyngeal delivery by mastication and mixing with saliva. This is a highly coordinated activity that is rhythmic and controlled to prevent injury to the tongue. The tongue is elevated toward the palate by the combined actions of the digastric, genioglossus, geniohyoid, and mylohyoid muscles. Intrinsic tongue muscles produce both the initial depression in the dorsum that receives the food and the spreading action that distributes the food throughout the oral cavity. The buccinator muscles hold food in the mouth in edentulous infants and help to generate suction in neonates. In this phase, the soft palate is against the tongue base, secondary to contraction of the palatoglossus muscles, which allows nasal breathing to continue [7, 41].

Table 20.2 Phases of normal deglutition

Phase	Activities	Time
Pre-oral (voluntary)	Food introduced into oral cavity	Varies; depends on substance
Oral phase (voluntary/ involuntary)	Bolus formation and passage to pharynx	Less than 1 s
Pharyngeal phase (involuntary)	Respiration ceases. Pharyngeal peristalsis. Epiglottis closes. Larynx closes, elevates, and draws forward. UES relaxes	1 s or less
Esophageal phase (involuntary)	Esophageal peristalsis. Opening of lower esophageal sphincter	8–20 s

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During the oral propulsive phase, the bolus is propelled into the oropharynx. The oral phase is characterized by elevation of the tongue and a posterior sweeping or stripping action produced mainly by the action of styloglossus muscles. This propels the bolus into the pharynx and triggers the "reflex swallow." The receptors for this reflex are thought to be at the base of the anterior pillars, but there is evidence that others exist in the tongue base, epiglottis, and pyriform fossae. Sensory impulses for the reflex are conducted through the afferent limbs of cranial nerves V, IX, and X to the swallowing center. Oral transit time is less than 1 s [7, 42].

The pharyngeal phase of deglutition is the most complex and critical. The major component of the pharyngeal phase is the reflex swallow. This results from motor activity stimulated by cranial nerves IX and X. Swallowing is elicited involuntary by afferent feedback from the oral cavity and has a duration of approximately 530 ms [1]. The reflex swallow may be triggered by a voluntary oral phase component or any stimulation of the afferent receptor in and around the anterior pillar [7]. Bolus passage through the pharynx is accompanied by soft palate elevation, lingual thrust, laryngeal elevation, and descent upper esophageal sphincter (UES) relaxation and pharyngeal constrictor peristalsis. The pharyngeal phase commences as the bolus head is propelled past the tongue pillars and finishes as the bolus tail passes into the esophagus [42]. Once it begins, the pharyngeal phase is very quick, 1 s or less [7]. It is characterized biomechanically by the operation of three valves and several propulsive mechanisms. During pharyngeal swallowing, respiration is inhibited centrally [43]. The larynx closes and the palate elevates to disconnect the respiratory tract. The UES opens to expose the esophagus. At the completion of the pharyngeal phase, the airway valves (larynx, palate) open and the UES closes so that respiration can resume [42].

Pharyngeal bolus transit occurs in two phases: an initial thrust phase and a mucosal clearance phase [44]. Bolus thrust, which propels most of the bolus into the esophagus, is provided by lingual propulsion, laryngeal elevation, and gravity. The tongue has been linked to a piston, pumping the bolus through the pharynx [45]. Patients with tongue impairment cannot generate large bolus driving forces despite an intact pharyngeal constrictor mechanism [46]. Laryngeal elevation creates a negative postcricoid pressure to suck the

oncoming bolus toward the esophagus, and the elevated larynx holds the pharyngeal lumen open to minimize pharyngeal resistance [45].

As the bolus enters the pharynx and is stripped inferiorly by the combined effects of gravity, the negative pressure mentioned above, and the sequential contractions of the pharyngeal constrictors, the soft palate moves against the posterior pharyngeal wall to close off the nasopharyngeal port. The bolus divides around the epiglottis, combines, and passes through the cricopharyngeal muscle or UES [7].

UES refers to the high-pressure zone located in between the pharynx and the cervical esophagus. The physiological role of this sphincter is to protect against reflux of food into the airways as well as prevent entry of air into the digestive tract [47]. Posteriorly and laterally, the cricopharyngeus muscle is a definitive component of the UES. The Cricopharyngeus muscle has many unique characteristics: it is tonically active, has a high degree of elasticity, does not develop maximal tension at basal length, and is composed of a mixture of slow- and fast-twitch fibers, with the former predominating. These features enable the cricopharyngeus muscle to maintain a resting tone and yet be able to stretch open by distracting forces, such as a swallowed bolus and hyoid and laryngeal excursion. The cricopharyngeal muscle, however, constitutes only the lower one-third of the entire high-pressure zone. The thyropharyngeus muscle accounts for the remaining upper two-thirds of the UES. The UES function is controlled by a variety of reflexes that involve afferent inputs to the motor neurons innervating the sphincter [47]. Based on functional studies, it is believed that the major motor nerve of the cricopharyngeal muscle is the pharyngoesophageal nerve. Vagal efferents probably reach the muscle by the pharyngeal plexus, using the pharyngeal branch of the vagus [48]. The superior laryngeal nerve may also contribute to motor control of the cricopharyngeal muscle [6]. Sensory information from the UES is probably provided by the glossopharyngeal nerve and the sympathetic nervous system. There is probably little or no contribution by the sympathetic nervous system to cricopharyngeal muscle control [48].

The relaxation phase begins as the genioglossus and suspensory muscle pulls the larynx anteriorly and superiorly. The bolus is carried into the esophagus by a series of contraction waves that are a continuation of the pharyngeal stripping action [7]. Proposed functions of the UES include prevention of esophageal distention during normal breathing and protection of the airway against aspiration following an episode of acid reflux [6, 48]. Qualitative abnormalities of UES have been documented in infants with reflux disease [49].

The esophageal phase occurs as the bolus is pushed through the esophagus to the stomach by esophageal peristalsis. Esophageal transit time varies from 8 to 20 s [26].

Dysphagia

Dysphagia is an impairment of swallowing involving any structures of the upper gastrointestinal tract from the lips to the lower esophageal sphincter [50]. Dysphagia in children is generally classified as either oral dysphagia (abnormal preparatory or oral phase) or pharyngeal dysphagia (abnormal pharyngeal phase).

Oral dysphagia is seen most commonly in children with neurodevelopmental disorders. Infants with oral dysphagia often have an impaired oral preparatory phase. These children typically demonstrate poor lingual and labial coordination, resulting in anterior substance loss and poor labial seal for sucking or removing food from a spoon. Other abnormal patterns include jaws thrust and tongue thrust on presentation of food. Oral dysphagia also may involve the oral phase of swallowing. Children with impaired oral phase function often have difficulty in coordinating the "suck, swallow, breathe" pattern of early oral intake, resulting in diminished endurance during oral feeds. Apraxia of oral swallow as well as reduction of oral sensation also is common. Other deficits include reduced bolus formation and transport, abnormal hold patterns, incomplete tongue to palate contact, and repetitive lingual pumping [26].

Oropharyngeal dysphagia results from either oropharyngeal swallowing dysfunction or perceived difficulty in the process of swallowing. Major categories of dysfunction are as follows: (1) an inability or excessive delay in initiation of pharyngeal swallowing, (2) aspiration of ingestate, (3) nasopharyngeal regurgitation, and (4) residue of ingestate within the pharyngeal cavity after swallowing. Each of these categories of dysfunction can be subcategorized using fluoroscopic and/or manometric data [29].

Clinical Signs/Symptoms

Clinical signs and symptoms of sucking/swallowing disorders in infants and children are listed in Table 20.3 [13].

Complications

Malnutrition

In the severely affected child with impaired swallowing, poor oral and/or pharyngeal function may lead to decreased energy intake as a consequence of prolonged feeding time and the inability to ingest adequate volumes, and malnutrition may result [6]. Malnutrition has many adverse effects. The most significant effects are on behavior and immune status. Malnutrition negatively influences immune status. This leads to recurrent infections that

Table 20.3 Clinical signs and symptoms of dysfunctional sucking/swallowing

Clinical signs
Failure to thrive
Meal-time distress
Refusing food
Nasal regurgitation
Wet or hoarse voice
Drooling
Spitting
Vomiting
Gastroesophageal or pharyngeal reflux
Symptoms
Oral-tactile hypersensitivity
Feeling of obstruction
Odynophagia
Atypical chest pain
Respiratory manifestations
Coughing
Choking
Stridor
Change in respiration pattern after swallowing
Apnea and bradycardia (predominantly in infants)
Noisy breathing after feeding
Chronic recurrent wheezing
Chronic recurrent bronchitis, pneumonia, and atelectasis

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increase caloric requirements but decrease intake, leading to worsening nutritional status. In addition, malnutrition causes behavioral apathy, weakness, and anorexia, which can all profoundly affect feeding, and secondarily, nutritional status. Thus, although malnutrition is often a direct result of poor feeding skills, it can also have compounding and even perpetuating effect on feeding problems in children [26].

Sialorrhea

Sialorrhea, or excessive drooling, is defined as the unintentional loss of saliva and other oral contents from the mouth. Drooling usually occurs in patients with neurologic disease complicated by abnormalities of the oral phase of deglutition. Clinical complications of drooling include soaking of clothes, offensive odors, macerated skin, and if "posterior" drooling occurs, aspiration [51].

Respiratory Complications

Respiratory complications of swallowing disorders include apnea and bradycardia, choking episodes, chronic or recurrent pneumonia, bronchitis, and atelectasis [52].

Apnea and bradycardia may result from stimulation of laryngeal chemoreceptors without evidence of aspiration or as a consequence of hypoxemia. Hypoxemia may result from the effects of direct aspiration on gas exchange, from apnea triggered by laryngeal and nasopharyngeal chemoreceptors, or in patients with compromised lung function as a result of normal decrease in minute ventilation that occurs with the suckle feeding [53–55]. Symptoms such as chronic recurrent coughing, choking, and postprandial congestion or wheezing generally indicate the occurrence of aspiration. Infants, especially premature infants, appear to be at an increased risk of respiratory disease from dysfunctional swallowing [9]. Clinical manifestations of dysfunctional sucking/swallowing in infants are primarily apnea and bradycardia during feeding, although chronic/recurrent respiratory problems (congestion, cough, and wheezing) are also seen [52]. Congested or noisy breathing during and following feeding is also a common complaint of parents of infants with dysfunctional swallowing. Dysphagia can also be an important but underrecognized cause of chronic/recurrent bronchitis, asthma, and pneumonia in infants [9].

Respiratory disease secondary to dysphagia in an older child is typically seen in a neurologically impaired host [56, 57]. Apnea and bradycardia are uncommon in older children. Bronchitis, pneumonia, atelectasis, and recurrent wheezing are more likely to be seen in this population. Feeding and swallowing evaluation should be considered in those with CNS injury affecting cranial nerve function and difficult-to-control chronic/recurrent bronchitis, wheezing, pneumonia, or asthma. Tracheobronchomalacia, a complication of chronic inflammation of the major airways, occurs commonly. Dysfunctional swallowing is also encountered in children with a tracheostomy. The tracheostomy may interfere with normal laryngeal function during swallowing and predispose to aspiration [50].

Aspiration may also occur in children with disorders of swallowing after an episode of gastroesophageal reflux; also, acid reflux may result in bronchospasm, pneumonia, or apnea [58, 59].

The most obvious sign that a person may have aspirated is the post-swallow cough, but in the swallowing-impaired children other more insidious indicators may be present. "Silent aspiration" with no clinical signs can account for over half of all cases of radiologically defined aspiration [50, 60].

Diagnosis

Feeding disorders and dysphagia in infants and children can be both physiological and behavioral in nature [61].

The evaluation of feeding and swallowing dysfunction is best performed as a multidisciplinary process with coordinated input from a variety of team members, including pedi-

atricians, pediatric gastroenterologists, developmental pediatricians, speech–language pathologists (SLP), occupational therapist, and pediatric dietitians [62]. The goals of this evaluation include the following: (1) ascertain whether oropharyngeal dysphagia is likely and identify the likely etiology, (2) identify structural etiologies of oropharyngeal dysfunction, (3) ascertain the functional integrity of the oropharyngeal swallow, (4) evaluate the risk of aspiration pneumonitis, and (5) determine if the pattern of dysphagia is amenable to therapy [63].

The investigation and management of swallowing disorders are summarized in Fig. 20.1 [13, 50].

History

A comprehensive history, obtained from individuals directly involved in caring for the child (e.g., parents, feeding specialist), is essential in evaluating children with swallowing disorders. The evaluation begins with a focused feeding history, including current diet, textures, and route and time of administration, modifications, and feeding position. Medical comorbidities that may affect swallowing need to be investigated.

Child's caretakers also should be questioned regarding associated symptoms, such as oral aversion, weak sucking, irritable behavior, gagging and choking, and disruptions in

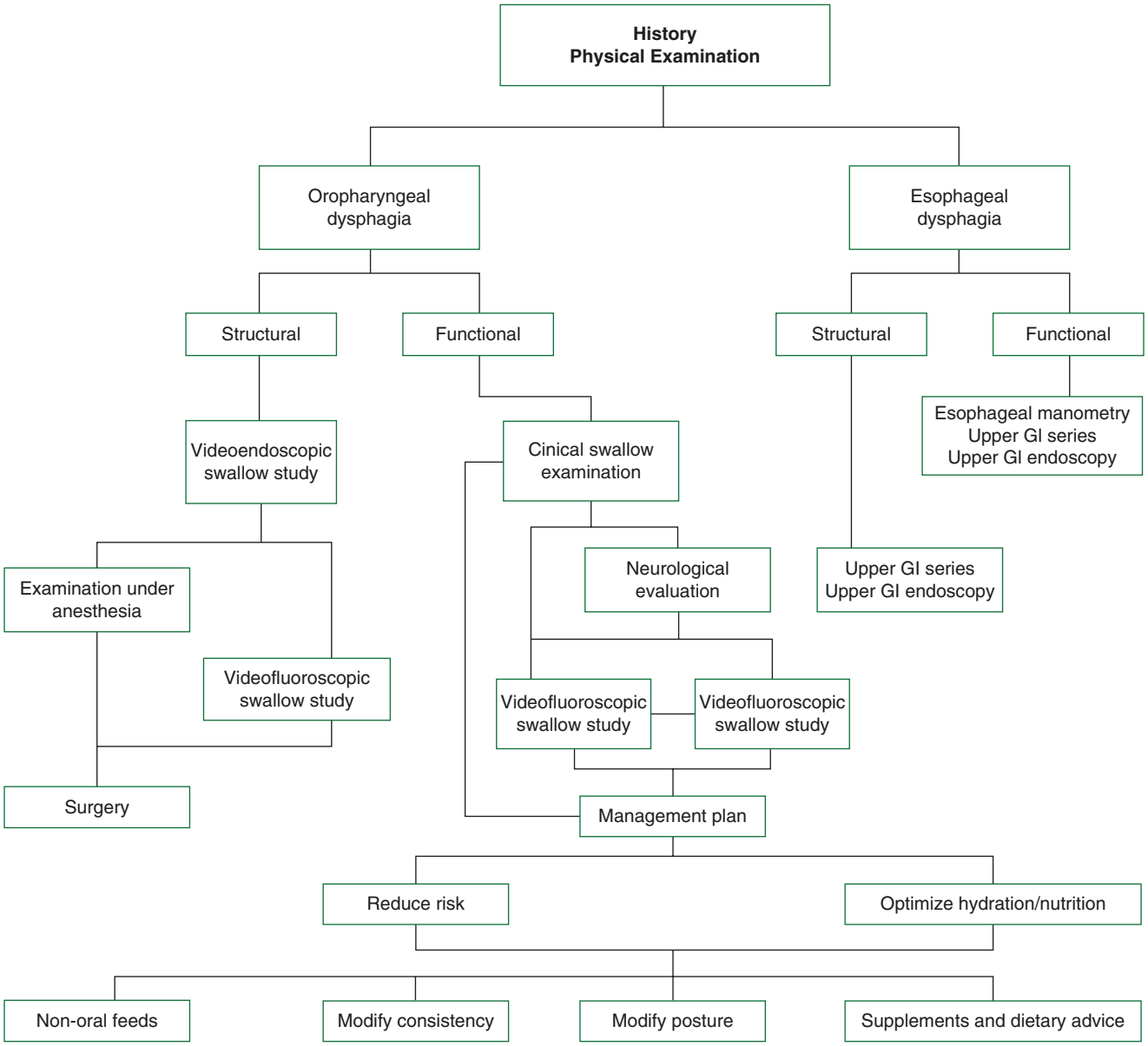

Fig. 20.1 Flowchart for the investigation and management of dysphagia in children. (Adapted by permission from BMJ Publishing Group Limited, from Ref. [50] and reprinted with permission from Ref. [13])

breathing or apnea. Postural or positional change during feeding may also be reported in children with dysphagia. Odynophagia and emesis may be related with pharyngeal and/or esophageal disorders. A history of recurrent pneumonia may indicate chronic aspiration; a history of stridor in relation to feeding may indicate a glottic or subglottic abnormality contributing to feeding disorders. Determining whether these symptoms occur before, during, or after the swallow helps localize the affected phase [27, 28].

In addition, nutritional and psychological assessment should be evaluated. Many patients with swallowing disorders have concurrent illness that may increase metabolic needs. Psychological assessments help to identify behavioral and parental factors that may be contributing to a feeding disorder. Psychosomatic causes of dysphagia should be considered in adolescents with dysphagia [7, 64, 65].

Physical and Clinical Evaluation

In dysphagic patients, physical examination aims to: (1) characterize the underlying systemic or metabolic disease when present; (2) localize the neuroanatomical level and severity of a causative neurological lesion when present; and (3) detect adverse sequelae such as aspiration pneumonia or nutritional deficiency [29].

There are four key questions that physicians and nurses in primary care can ask parents when an infant or young child presents at the office or clinic with parental concerns related to feeding [66].

The answers help determine whether a comprehensive clinical feeding and swallowing assessment is needed, even though the answers do not necessarily define the problem:

- How long do mealtimes typically take? If more than about 30 min on any regular basis, there is a problem. Prolonged feeding times are major red flags pointing to the need for further investigation.
- Are mealtimes stressful? Regardless of descriptions of factors that underlie the stress, further investigation is needed. It is very common for parents to state that they "just dread mealtimes."
- Does the child show any signs of respiratory stress? Signs may include rapid breathing, gurgly voice quality, nasal congestion that increases as the meal progresses, and panting by an infant with nipple feeding. Recent upper respiratory illness may be a sign of aspiration with oral feeds, although there may be other causes.
- Has the child *not* gained weight in the past 2–3 months? Steady appropriate weight gain is particularly important in the first 2 years of life for brain development as well as overall growth. A lack of weight gain in a young child is like a weight loss in an older child or adult.

Following the physical examination, the focus is on the upper aerodigestive tract, beginning with an examination for structural and functional abnormalities. Oral cavity anatomic abnormalities, such as ankyloglossia, cleft lip or palate, or macroglossia, need to be excluded [7]. The palatal gag is perhaps the most assessed reflex and should be evaluated [51]. A hyperactive gag can result in significant feeding difficulties, and in the past an absent gag reflex was considered as an indication to stop oral feeding [9, 56].

It is critical that observation of the feeding process be included [62]. This part of the examination is best performed in conjunction with a feeding and swallowing specialist, such as a SLP or an occupational therapist. This examination includes assessments of posture, positioning, patient motivation, oral function, efficiency of oral intake, and clinical signs of safety. During the feeding trial, the presence of abnormal movements such as jaw thrust, tongue thrust, tonic bite reflex, and jaw clenching is noted. A variety of therapeutic positions, techniques, and adaptive feeding utensils may be used [6, 27].

A variety of assessment scales may be used to detail and quantitate results of the swallowing evaluation. However, all assessments are based on similar observations of feeding structure and function [67].

The clinical evaluation differs according to the patient age. In infants, from birth till 1 year of age, including those in the Neonatal Intensive Care Unit (NICU), the evaluation includes the assessment of prefeeding skills, the readiness for oral feeding, and the evaluation of breast- and bottle-feeding ability. For toddlers (ages 1–3 years) and pre-school/school-age children/teenagers (ages 3–18 years), the evaluation may also include a review of any past diagnostic test and any current treatment programs, the assessment of current skills and limitations at home and at school, patient acceptance of liquids and solid food, and the grade of independence or need for supervision and assistance during the feeding [68].

Usually, a careful developmental, medical, and feeding history provides clues to the diagnosis that guide the selection of further diagnostic tests. Only after all reasonable physical causes have been ruled out, should a feeding or swallowing disorder be attributed to a purely behavioral cause [7].

Diagnostic Tests

Radiographic Assessment

The Videofluoroscopic Swallow Study (VFSS) also known as modified barium swallow, represents the gold standard method for evaluation of children with swallowing disorders [69]. A videofluoroscopic swallow study is ideally performed

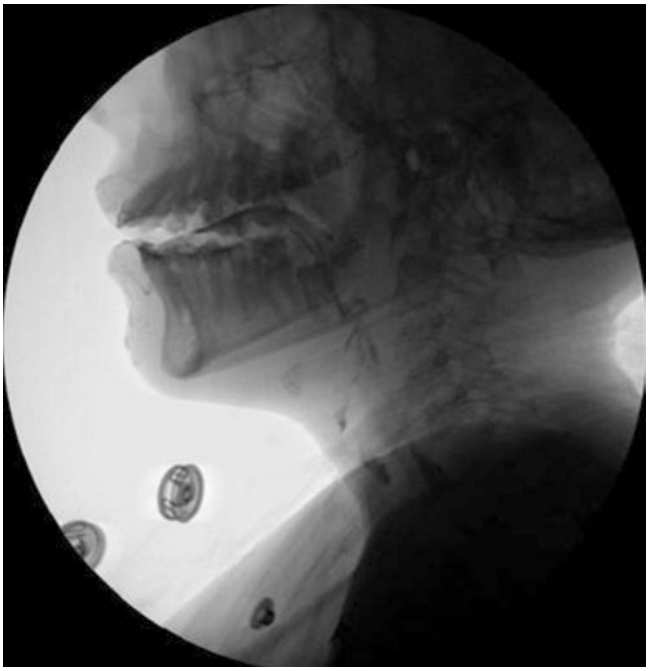

Fig. 20.2 Lateral fluoroscopic projection of a child showing contrast material in the valleculas, pyriform sinuses, laryngeal vestibule, and esophagus

by a consultant radiologist and specialist and the SLP [70]. A series of swallows of varied volumes and consistencies of contrast material are imaged in a lateral projection, framed to include the oropharynx, palate, proximal esophagus, and proximal airway. Studies are recorded on videotape to permit instant replay, in slow motion if necessary, and examination of both the presence and mechanism of swallowing dysfunction. As such, the videofluoroscopic study provides a dynamic view of all the stages of swallowing (oral preparatory, oral, pharyngeal, and upper esophageal), therefore assuring evidence of all four categories of oropharyngeal swallowing disorders: (1) inability or excessive delay in initiation of pharyngeal swallowing, (2) aspiration of ingestate, (3) nasopharyngeal regurgitation, and (4) residue of ingestate within the pharyngeal cavity after swallowing. Furthermore, the procedure allows for testing of the efficacy of compensatory dietary modifications, postures, swallowing maneuvers, and facilitatory techniques in correction or observed dysfunction. Generally, the videofluoroscopic evaluation is completed by esophagography to evaluate the esophageal phase of deglutition (Fig. 20.2) [29]. A nasogastric tube does not alter the findings of videofluoroscopic swallowing study and does not increase the risk of aspiration; however, it might increase the incidence of respiratory compromise when aspiration is present [71]. The disadvantage of the technique is related to the ionizing radiation exposure that imposes a time-limited examination and the need for patient cooperation. In order to ensure that the radiation dosage amount is as low as reasonably achievable (ALARA) [72], the radiologist and the SLP work together following the International Commission on Radiological Protection (ICRP) guidelines [68].

Fiberoptic Endoscopic Examination

Pediatric fiberoptic endoscopic examination (FEES) is a relatively new diagnostic method to add to the current armamentarium of techniques for evaluating dysphagia and/or aspiration. FEES is performed by passing a flexible laryngoscope into the oropharynx after anesthetizing the nares and nasopharynx [73]. It allows to diagnose many of the laryngeal disorders that may affect the child, while at the same time evaluating the swallowing mechanism itself. The procedure involves five components: assessment of anatomy (as it affects swallowing), evaluation of movement and sensation of critical structures, assessment of secretion management, direct assessment of swallowing function for food and liquid, and patients' response to therapeutic maneuvers. Compared to the VFSS, FEES does not imply radiation exposure to the infant as well as no barium ingestion. In addition, FEES allows the visualization of the pharyngeal and laryngeal anatomy and assesses secretion management. Moreover, the procedure is less expensive and it can be performed at the bedside whether the patient transportation is not possible or difficult. FEES can be repeated more frequently to assess patient improvement as there is no risk related to radiation exposure and it can be also used to assess breastfeeding. Unlike the VFSS, FEES does not allow the visualization of the oral phase and the contractions of the tissue surrounding the end of the endoscope during the swallow cause a temporary condition called "white-out" where the passage of the bolus and movement of the structures cannot be observed. In experienced hands, this test can be performed in children with minimal discomfort. Possible described adverse effects are epistaxis, laryngospasms, reaction to topical anesthesia and vasovagal response [74–76]. A variation of the FEES, the fiberoptic endoscopic evaluation of swallowing with sensory testing (FEESST), utilizes an air pulse stimulus of mechanoreceptors within the larynx to study the laryngeal adductor response (LAR). The study can be performed safely in premature infants, in children, and in adults [77].

Ultrasonography

Ultrasound imaging has been used to a limited extent in the assessment of oral phase dysphagia. Using a transducer positioned in the submental region, ultrasonography allows observation of the motion of structures in the oral cavity,

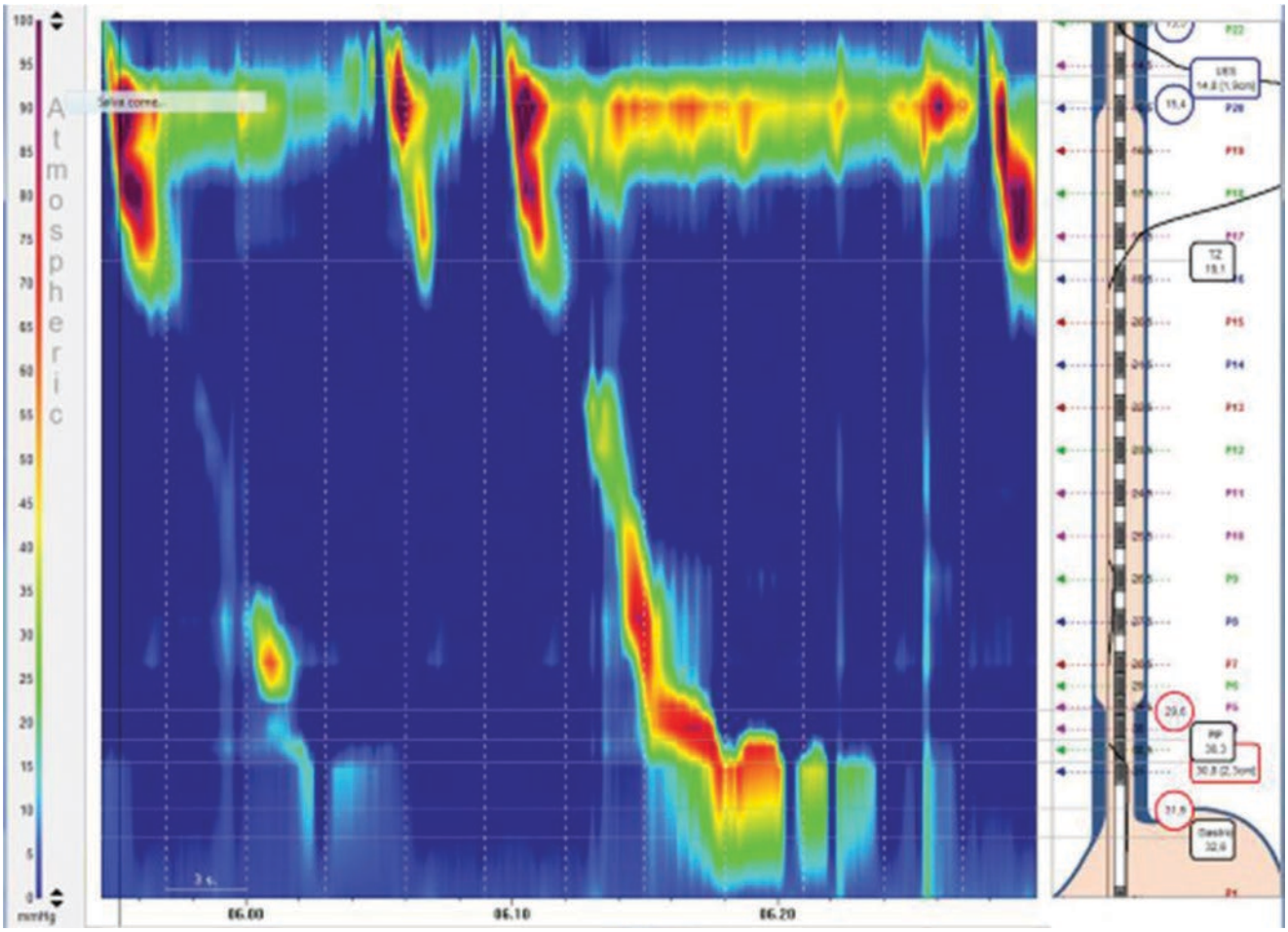

Fig. 20.3 Esophageal pressure topography plotting: complete peristaltic chain studied with a 21-lm catheter. The three *inter-segmental* troughs are indicated in the figure, and the pressure amplitudes represented by the isobaric contour regions are shown in the *color legend* (in mmHg above gastric baseline pressure; pressures below the first isobaric contour are shown in *blue*). SW swallow

such as the tongue and floor of the mouth during feeding and deglutition, but lacks sensitivity in visualizing pharyngeal motion and determining whether aspiration has occurred. Ultrasonography may be particularly useful in distinguishing an infant's inability to latch on from maternal factors contributing to feeding difficulties [14]. Unfortunately, laryngeal penetration and aspiration are not easily detected because of the shadows cast by the laryngeal structures [9, 34, 78].

Pharyngeal Manometry

Intraluminal manometry, performed using a transnasally positioned manometric assembly, can quantify the strength of pharyngeal contraction, the completeness of UES relaxation, and relative time of these two events. Most studies indicate that the manometry of the UES and pharynx provides useful information primarily in patients who have symptoms of oropharyngeal dysfunction. The coordination of muscle activity at various levels can be obtained by simultaneous recording of pressure in the pharynx, at the level of cricopharyngeus, and in the esophagus. Anatomic references are not available with this technique [49, 79]. Recently, manometry equipment has evolved to allow more precise and detailed evaluation of esophageal function with high-resolution manometry and esophageal pressure topography plotting [80] (Fig. 20.3).

Scintigraphy

Scintigraphy is a radionuclide evaluation using Technetium-99m-labeled sulfur colloid mixed in the infant's formula. It has been proposed as an alternative and perhaps more sensitive way of quantifying aspiration, transit times, gastroesophageal reflux, and pharyngeal residue. Based on a case report, the radionuclide salivagram has also been used to document aspiration of saliva. The major limitations of this

technique are the poor definition of the anatomy and the poor sensitivity for detecting aspiration during swallowing in children with prior aspiration problems. At the present, the use of this technique in pediatric age is limited [76, 81].

Treatment Options

Optimal management strategies are critical for infants and children with feeding and swallowing problems. According to the World Health Organization's International Classification of Functioning, Disability, and Health (ICF) framework, the main treatment goal should aim to promote a functional mealtime experience for children and families, facilitating the patient participation by ensuring a safe and efficient feeding [68]. The management of swallowing dysfunction involves a team approach. Individuals involved in addition to the medical team include a swallowing expert (speech–language pathologist or occupational therapist), a nutritionist, and the family. Since swallowing abnormalities arise from a diverse group of underlying disorders, management techniques must be individualized. This heterogeneity is also reflected in the fact that patients have different potentials to recovery [6].

Although total oral feeding may not be a realistic goal, it is the universal hope of caregivers. Professionals are obligated to point out prerequisites for oral feeding and to discuss the probability that an in individual child may reach the goal. These management decisions are typically made on the basis of clinical observations and assessments. In addition, important information is obtained through an instrumental assessment by videofluoroscopic swallows study. A methodical videofluoroscopic swallowing study: (1) defines the anatomy of the oropharynx; (2) detects dysfunction as evident by aspiration, poor clearance, or poor control of the bolus; (3) determines the mechanism responsible for the dysfunction; and (4) examines the short-term effects of the therapeutic strategies designed to eliminate or compensate for that dysfunction [82]. Management decisions may incorporate nutritive recommendations, medical and surgical decisions, position guidelines, oral-motor/swallow practice, and behavioral intervention [83].

The clinical and instrumental evaluation of children with sucking and swallowing disorders should allow for the recognition of treatable anatomic or inflammatory lesions.

A child may refuse to eat even if his anatomic abnormality has been corrected because of learned aversion to feeding. Behavior therapy often can overcome this type of conditioned food refusal [7, 65].

Various therapeutic approaches may improve the efficiency and safety of feeding. Management techniques involve devising compensatory strategies to minimize swallowing-related complications [84].

Table 20.4 Swallowing strategies for pediatric dysphagia

Behavioral training	
Dietary modification	Thickened liquids
	Thin liquids
Proper intrabolus placement	Modify feeding utensils and bolus presentation
Swallowing exercises	Supraglottic swallow
	Supersupraglottic swallow
	Effortful swallow
	Mendelsohn maneuver
Modification in body tone posture seating and positioning	Head tilt
	Chin tuck
	Head rotation
	Lying on side, elevation
Suckle-feeding-valved feeding bottle	–
Cricopharyngeal myotomy	–
Facilitatory techniques	Biofeedback
	Thermal stimulation
	Gustatory stimulation
Provide alternate means of enteral nutrition	Nasogastric feeding
	Gastrostomy tube (surgical or endoscopic)

Reprinted with permission from Ref. [13], Table 15.4, p. 244

These include changing the textures of foods; pacing of feeding; changing the bottle or utensils; and changing the alignment of the head, neck, and body with feeding (Table 20.4) [13, 80].

Frequently, children with severe anatomic disorders but normal neurological function develop their own adaptive strategies to allow for safe oral feeding. Unfortunately, many children with feeding disorders have non-correctable neurologic or anatomic abnormalities that make oral feeding difficult or unsafe. Some patients cannot obtain adequate nutrition by mouth because of a risk for aspiration. Thus, supplying a portion of patient's nutrition by nasogastric or gastrostomy feeding may be beneficial [7]. For those children who have been intubated, management includes teaching techniques that will facilitate transitioning from non-oral to oral feeding. However, there is little evidence that non-oral feeding reduces or eliminates the risk of aspiration [85–87].

The strongest evidence-based recommendation that can be made pertains to diet modification. Furthermore, the literature provides reasonable evidence of the plausibility of swallowing therapy but minimal evidence of efficacy. Nonetheless, although no hard evidence supports its efficacy, the available data are inconclusive and swallowing therapy has not been proven ineffective. Thus, the current weight of opinion, combined with the convincing demonstration of biological plausibility for specific techniques and the consistency of low-grade evidence, is the basis to recommend that swallowing therapy should be used. Large-scale randomized, controlled trials are needed to clarify the current recommendations [29].

Prognosis

Prognosis depends on underlying conditions that predispose to impaired sucking and swallowing. However, the early recognition of feeding problems, diagnosis of underlying disorders, and appropriate intervention improve outcomes for the child and the family.

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Additional Educational Resources:

Resource center: The Dysphagia Research Society is organized exclusively for charitable, educational, and scientific purposes (http://www.dysphagiaresearch.org/) – this multidisciplinary website has a wealth of information on dysphagia, references to texts, archives, and links to other related sites; user friendly and very comprehensive.

Defecation Disorders in Children: Constipation and Fecal Incontinence

21

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Part 1: Constipation

Definition

Constipation is a bothersome problem for many children and parents. It is characterized by infrequent, large, hard, or painful stools, with or without fecal incontinence, and may be accompanied by abdominal pain. In approximately 95% of children, no organic cause is found: these children suffer from functional constipation, which is one of the most common functional gastrointestinal disorders in children [1, 2]. Currently, the most widely accepted criteria to diagnose functional constipation in children are the Rome IV criteria (Table 21.1) [3, 4]. Compared to the Rome III criteria, the duration of complaints in order to diagnose pediatric functional constipation was shortened from 2 to only 1 month [5, 6]. In addition, a differentiation was made for children who are or are not toilet trained. These changes were necessary to facilitate early treatment and because most toddlers younger than 2.5 years of age are not (yet) toilet trained.

Table 21.1 Rome IV criteria for functional constipation [3, 4]

Diagnostic criteria for infants up to 4 years of age:
Must include at least two of the following for a minimum of 1 month: 1. Two or fewer defecations per week 2. History of excessive stool retention 3. History of painful or hard bowel movements 4. History of large-diameter stools 5. Presence of a large fecal mass in the rectum In toilet-trained children, the following additional criteria may be used: 1. At least one episode/week of incontinence after the acquisition of toileting skills 2. History of large-diameter stools that may obstruct the toilet
Diagnostic criteria for children from 4 years of age:
Must include at least two of the following occurring at least once per week for a minimum of 1 month with insufficient criteria for a diagnosis of irritable bowel syndrome: 1. Two or fewer defecations in the toilet per week in a child of a developmental age of at least 4 years 2. At least 1 episode of fecal incontinence per week 3. History of retentive posturing or excessive volitional stool retention 4. History of painful or hard bowel movements 5. Presence of a large fecal mass in the rectum 6. History of large-diameter stools that can obstruct the toilet After appropriate evaluation, the symptoms cannot be fully explained by another medical condition

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The use of these diagnostic criteria is important because using a single clinical feature to define functional constipation, such as low bowel frequency, can be misleading. It has been shown that around 0.4–20% of otherwise healthy children have at least one feature of the Rome criteria for functional constipation [7, 8]. Furthermore, bowel frequency is known to vary in different regions of the world depending on diet, genetics, and environmental factors [7, 9]. Therefore, it is imperative that the clinician's perspective is flexible and that he or she understands the abnormalities in bowel habits in context of local and patient variables. In addition, the use of diagnostic criteria is essential for research purposes to select subjects and evaluate treatment success [10].

Several studies have assessed the diagnostic capability of the Rome IV criteria to identify functional constipation in children. Studies comparing the prevalence of functional constipation according to Rome III and Rome IV criteria found no significant differences in prevalence [1, 11, 12]. An Italian study including over 200 children and adolescents, aged 0–17 years, found good agreement between the two criteria for diagnosing functional constipation [13].

Prevalence

Constipation is a global public health problem. Reported prevalence ranges from 0.5% to 32.2%, with a worldwide pooled prevalence of 9.5% (95% CI 7.5–12.1) [14]. Studies from Europe show a prevalence ranging from 11.7% to 18.2% [13, 15], and a recent North-American study found functional constipation to be one of the most prevalent functional gastrointestinal disorders among both toddlers (18.5%), and children and adolescents (14.1%) [1].

Prevalence in Latin America range from 6.4% to 10.7% [11, 16], and studies in Asia show a wide range in prevalence of functional constipation from 8.0% in Sri Lanka, 18.3% in Indonesia, to 32.3% in Taiwan [17–19]. Studies evaluating the prevalence of constipation in African countries remain scarce; one study in Nigeria reports a high prevalence of 27% [20]. The differences in reported prevalence need to be interpreted with caution, as the wide variations found may partly be due to differences in definitions used, differences in age groups included, and heterogeneity of survey methods. However, all together these data underscore the magnitude of the disease burden of children with constipation all around the globe.

Physiology of Defecation

Human defecation is the act of eliminating waste materials (feces) from the digestive tract and involves a coordinated number of neurological, muscular, physiological, and behavioral functions. In the colon, intraluminal contents are extracted and the remnant is gradually propelled through the colon. In the transverse colon, pacemaker cells initiate propagation waves in both retrograde (toward the ileum) and antegrade (toward the anus) direction. A few times a day large contractions of colonic smooth muscle result in the antegrade propagation of intraluminal contents. These contractions are called high amplitude propagating contractions (HAPCs) [21]. HAPCs can be propagated from cecum to rectum and are associated with movement of colonic content and defecation [22–24]. Colonic motor activity has a circadian pattern and increases after awakening and eating a meal [22]. Eventually, after propagation, the intraluminal contents arrive in the rectum which acts as a reservoir. During rest, the anal canal is closed by the tonic activity of the internal and external anal sphincters, together with the anal cushions. Further filling of the rectum leads to distension of the rectal wall, which subsequently triggers a transient relaxation of the internal anal sphincter, called the rectoanal inhibitory reflex (RAIR). The RAIR is controlled by the enteric nervous system and partly regulated by the sacral cord [25]. The relaxation of the internal anal sphincter allows descent of rectal contents into the upper anal canal, resulting in a perception of their nature and a possible urge to defecate. The levator ani, puborectalis, and external anal sphincter then remain in a state of continuous contraction (the postural reflex) to keep the feces in the anal canal [25]. Finally, at an appropriate time, the external anal sphincter and pelvic floor muscles relax, and the abdominal wall contracts to empty the anal canal and distal rectal contents.

Risk Factors

Table 21.2 shows the risk factors for developing constipation in children. In contrast to adult studies which show constipation to be more prevalent among females, a recent meta-analysis found no difference in the prevalence of constipation between boys and girls [14, 26]. The same meta-analysis found evidence for associations between the presence of constipation and geographical location, psychological factors, and dietary habits. Geographically, children living in the Americas and Europe seem to have a higher risk of constipation compared to children living in Asia [14].

Psychological stress is another risk factor that predisposes children to develop constipation. The origin of the effect of psychological stress may lay in line with the brain-gut axis described in functional abdominal pain disorders [4]. The brain-gut axis describes the continuous interaction between brain and gut in regulating digestive and immune processes, and the coordination of the physical and emotional state of

Table 21.2 Putative risk factors for childhood constipation

Category	Risk factor
Lifestyle	Poor sleep Decreased physical activity
Dietary	Low fiber intake Low intake of fruit and vegetables Consumption of junk food Not having regular meals with parents Cow's milk protein allergy
Psychological	Home-related stresses School-related stresses Adverse life events including abuse Subjected to bullying Anxiety Depression Psychological maladjustment Autistic spectrum disorders
Social	Lower social class Hostile and aggressive family environment

the entire organism (sleep, hunger, stress) with the activity in the gastrointestinal tract [27]. Common school and home-related stressful life events, as well as adverse life events, such as abuse, predispose children to develop constipation [14, 18, 28–30]. However, in a recent study including Palestinian refugee preschool children, the prevalence of constipation was comparable to prevalence rates among older children worldwide. In that study, stressful life events and trauma exposure did not seem to play an important role in the development of functional constipation [31]. Irregularities in daily life, like absence of parents at home, not having regular meals with parents, and inadequate sleep also seem to be associated with the development of constipation [32, 33]. In addition, the refusal of having bowel movements at school toilets, and bullying are important risk factors to develop constipation [32, 33]. The association between constipation and obesity remains controversial as studies report conflicting data [34]. Decreased physical activity and less outdoor play have been associated with the presence of constipation and may be the reason for conflicting associations in obese children [35–38]. When evaluating dietary factors, low consumption of fruits and vegetables [32, 33, 36, 38], low consumption of fiber [36, 39], and frequent consumption of fast foods [32] are associated with constipation. Studies have suggested that a decreased fluid or fiber intake and cow's milk allergy may be causes of constipation [40–42]. The data on these topics however remain scarce. In infants, breastfeeding seems to be protective for the development of constipation [38, 43]. No association was found between cesarean section and the development of constipation in infants [44]. A randomized field trial performed in Brazil found that promoting healthy infant feeding practices was effective in reducing the prevalence of constipation among children at 6 years of age [45].

Quality of Life

Children with constipation have poor health-related quality-of-life (HRQoL) scores in all domains, namely social, school, physical, and emotional functioning [46, 47]. Children with constipation report even lower HRQoL scores compared to children suffering from organic diseases such as gastroesophageal reflux, and inflammatory bowel disease [47]. Surprisingly, a recent meta-analysis found no evidence for a difference in HRQoL between children with and without fecal incontinence [46]. Factors thought to be responsible for the impaired HRQoL include the chronic nature of the condition, psychosocial comorbidities, and adverse social effects of symptoms like school absenteeism, fecal incontinence, or clogging of toilets at school [48–51]. In addition, children with constipation often suffer from an array of somatic symptoms which are in turn associated with lower HRQoL [50].

Healthcare Burden

Constipation is a leading cause for medical consultation in children. A prospective study in the United States has shown that from childhood to early adulthood, individuals with functional constipation have higher medical care use, including high outpatient costs and emergency department utilization compared to matched controls [52]. The use of medical resources by children with constipation is substantially higher than that of other chronic episodic conditions such as asthma (seven times) and migraine headaches (three times) [52]. In an analysis of national emergency department sample records for emergency room visits of children of all ages across the United States, 17 per 100.000 emergency department visits were visits because of fecal impaction [53]. Furthermore, children with constipation are noted to have higher number of days of school absenteeism [51, 54] and children with fecal incontinence show poorer school performance [51]. Implications of these findings on education of children are substantial as poor education invariably leads to poor earning capacity and is associated with poorer health [55].

Pathophysiology

In more than 95% of pediatric cases, no underlying organic cause for constipation can be found, and these children are therefore diagnosed with functional constipation [2]. In the 5% of children with an organic cause for constipation, the etiology varies from Hirschsprung's disease, anorectal malformations, neuromuscular disease, to metabolic and endocrine disorders. A list of organic causes is provided in Table 21.3.

Table 21.3 Organic causes of constipation in children

Anatomical causes
Anorectal malformation
Intestinal obstruction
Metabolic and endocrine causes
Celiac disease
Cystic fibrosis
Dehydration
Diabetes mellitus
Hypo- or hypercalcemia
Hypokalemia
Hypothyroidism
Neurogenic conditions
Hirschsprung's disease
Autonomic neuropathy
Botulism
Chagas disease
Myelodysplasia
Myelomeningocele
Myotonic dystrophy
Neurofibromatosis
Neuronal intestinal dysplasia
Pseudo-obstruction (e.g., myopathies, mitochondrial disorders)
Spinal cord abnormalities (e.g., tethered cord)
Other causes
Starvation (secondary to anorexia nervosa)
Depression
Immobility
Medications (e.g., opiates, anticholinergics, antidepressants, chemotherapy)
Sexual abuse

The pathophysiological mechanisms of functional constipation in children are multifactorial and remain incompletely understood. However, available studies on physiology of the colon and rectum have shed some light upon the subject.

Infants and Young Children

Especially in young children, stool withholding is the major contributing factor in the development of constipation. Some studies found that even over 90% of constipated young children show withholding behavior [56–58]. Typically, when a child feels the urge to pass stools, he or she tightens gluteal muscles and stands on tip toe to prevent the stool to pass. This behavior may be the result of a prior painful or frightening experience of defecating, strict early toilet training, stubbornness, and/or concentration on other, more fun activities than going to the bathroom. During the process of withholding stools, the rectum compliance increases, i.e., the rectum has a greater capacity to distend and relaxes more in response to distension [59]. Because of the increase of rectal compliance, larger stool volumes are required to reach the intrarectal pressure which triggers an urge to defecate. With the retention of stool and the continuous absorption of water by colonic and rectal mucosa, the stool becomes dry, hard, and (more) difficult to pass. Passing a painful stool then leads to an anticipation of pain which in turn triggers withholding behavior. The child gets into a vicious cycle of delaying defecation, leading to prolonged retention of stool, leading to dilation of the rectum and a delayed urge sensation, leading to further retention of stools, which become hard, large, and painful, resulting in more anticipated pain triggering withholding behavior, etc. In accordance with this developed aversion toward defecation, a self-efficacy scale has been developed to measure the child's confidence and motivation in the act of defecating [60]. Self-efficacy improved in children after their first clinic visit, and treatment success was associated with further improvement of self-efficacy [61]. Self-efficacy may have improved because of treatment success or may play a role in achieving treatment success which would make it an interesting therapeutic target. In addition to this intentional or subconscious withholding of stools when feeling an urge to defecate, a subset of children may "teach" themselves to withhold their stool even during defecation. The act by which during defecation a child contracts instead of relaxes his or her pelvic floor and external anal sphincter is called dyssynergic defecation, anismus, or pelvic floor dyssynergia. This paradoxical contraction may be responsible for further hampering defecation and is found in about 40% of children with constipation [62, 63]. Some suggest this contraction is the manometric equivalent of stool withholding [64].

Children and Adolescents

For older children, stool withholding may play a less important role, and other contributing factors have been identified in the development of constipation. In a subset of children, local colonic factors may play a role in the development of constipation such as delayed intraluminal colonic transport by decreased (segmental) propagation, which may result in delayed colonic transit times [65, 66]. With the use of colonic manometry, several abnormalities have been identified in children with constipation, including reduced frequency or premature termination of HAPCs, or abnormal colonic response to stimuli, such as a meal [67]. Children with intractable constipation may have a reduced number of postprandial retrograde cyclic propagating motor patterns and have an increased incidence of spontaneous long-single motor patterns [68]. A total absence of high amplitude propagating contractions may also be found in children with intractable constipation [69]. These factors may contribute to poor propulsion of fecal material along the colonic lumen resulting in symptoms of constipation.

Rectal sensitivity to oncoming fecal matter is crucial for normal rectal function. There is a subset of children with constipation who demonstrate poor rectal sensation [70]. Furthermore, several studies in children have shown increased rectal compliance [63, 71] and a megarectum [72]. These factors are closely interrelated and lead to attenuation

of rectal sensation and lack of desire to evacuate, leading to low bowel frequency.

Other psychological factors may also play a role. A study investigating differences in sensory patterns found that children with chronic constipation showed significantly higher sensory scores than a matched normative sample [73]. The authors hypothesize that children with high sensitivity may be avoidant of toileting and, therefore, experience higher levels of fecal retention. The effect of sensory responsiveness on the timing of defecation may also explain the associations between constipation and both internal and external behavior problems and behavioral disorders (such as autism spectrum disorder or attention-deficit/hyperactivity disorder) [74–77]. This may play a more pronounced role in older children, as a study has shown that children with later onset of symptoms were more likely to have behavioral or developmental issues [78]. In addition, parents may influence the development or continuation of constipation in their children. Studies have shown that parental personality, psychological and physical health, and child-rearing practices differ significantly between parents of children with functional constipation and parents of control subjects [79, 80]. High levels of frustration or irritability toward the child were shown to have a negative effect on fecal incontinence symptoms. This emphasizes the importance of educating the parents about functional constipation and the effect of their parenting practices.

The gut microbiome may also play a role in the pathophysiology of constipation within the brain-gut axis via endocrine, immune, and neural pathways [81]. Small studies have found differences in microbiota between healthy individuals and children and adults with functional constipation; however, the reported characteristics of microbiota related to functional constipation differ among studies [82–84]. Next to a role for the microbiome via the brain-gut axis, gut microbiota may modulate gastrointestinal motility as reported in animal studies, or by metabolites and fermentation products having osmotic effects and causing increased gas production [85, 86]. The microbiome may be affected by the diet, which is also thought to be a factor in the pathophysiology of constipation.

Final Pathway for Both Age Groups

Pathophysiological mechanisms described for both age groups, including important risk factors described earlier, are shown in Fig. 21.1. These combined factors lead to retention of stools in the rectum and colon. Since colonic and rectal mucosas are designed to absorb water, stool becomes dry, hard, and large. Accumulation of feces, leading to dilatation of the colon is associated with premature termination of HAPCs [65], and mechanical dilation of the rectum inhibits motor function of the proximal and distal hemi-colon through reflex mechanisms [88, 89]. In addition, accumulation of feces may elongate the colon [90]. This in turn leads to the release of nitric oxide by activating mechanosensory and myenteric descending neuronal nitric oxide synthase. Nitric oxide inhibits action potential firing in other myenteric sensory neurons driving peristaltic nerve circuits, inhibiting colonic contractile activity (occult reflex), thereby seriously hampering evacuation [91, 92]. It has been shown that children with increased rectal wall compliance have prolonged colonic transit time which further strengthens the possibility of occult reflex [71]. However, even though increased rectal compliance is found to be associated with more severe symptoms, it does not predict treatment success or change after successful treatment [70]. Interactions of these inextricably linked mechanisms in a complex manner, rather than in isolation, lead to generation and propagation of symptoms in children with constipation.

Clinical Evaluation

The most important tool in diagnosing defecation disorders in children is the clinical evaluation. A thorough history and physical examination, including rectal examination when only one symptom of the Rome IV criteria is present, can be sufficient to identify functional defecation disorders, exclude possible organic causes, and recognize complications. During history taking and physical examination, one should always be aware of alarm symptoms of constipation indicating organic causes of constipation or signs of physical abuse (see Box 1).

Clinical History

Although the presenting features are obvious in most children, in some children clinical features may be subtle. Therefore, a high degree of suspicion is essential during history taking. Onset and duration of symptoms need to be clarified first. A very early onset in infancy suggests the possibility of organic diseases such as Hirschsprung disease, anorectal malformations, and metabolic diseases. However, breastfeeding may be responsible for a wide range in defecation patterns which should not be directly considered abnormal [93–95]. Details of bowel habits are the cornerstone in diagnosing constipation. The use of validated stool scales for infants (Amsterdam stool scale and Brussels Infant and Toddler Stool Scale) and children (modified Bristol stool scale) help to obtain more accurate description of stools [96–98]. Apart from gathering information on bowel habits, it is important to look for other gastrointestinal and urinary symptoms, the effect of constipation symptoms on daily life, and to identify risk factors that may contribute to persistence of symptoms. Moreover, abdominal pain is noted in 10–70% of children with constipation. When the abdominal pain does not improve after resolution of constipation symptoms, the diagnosis of irritable bowel syndrome (IBS) should be considered [4]. Although the Rome criteria consider IBS and functional con-

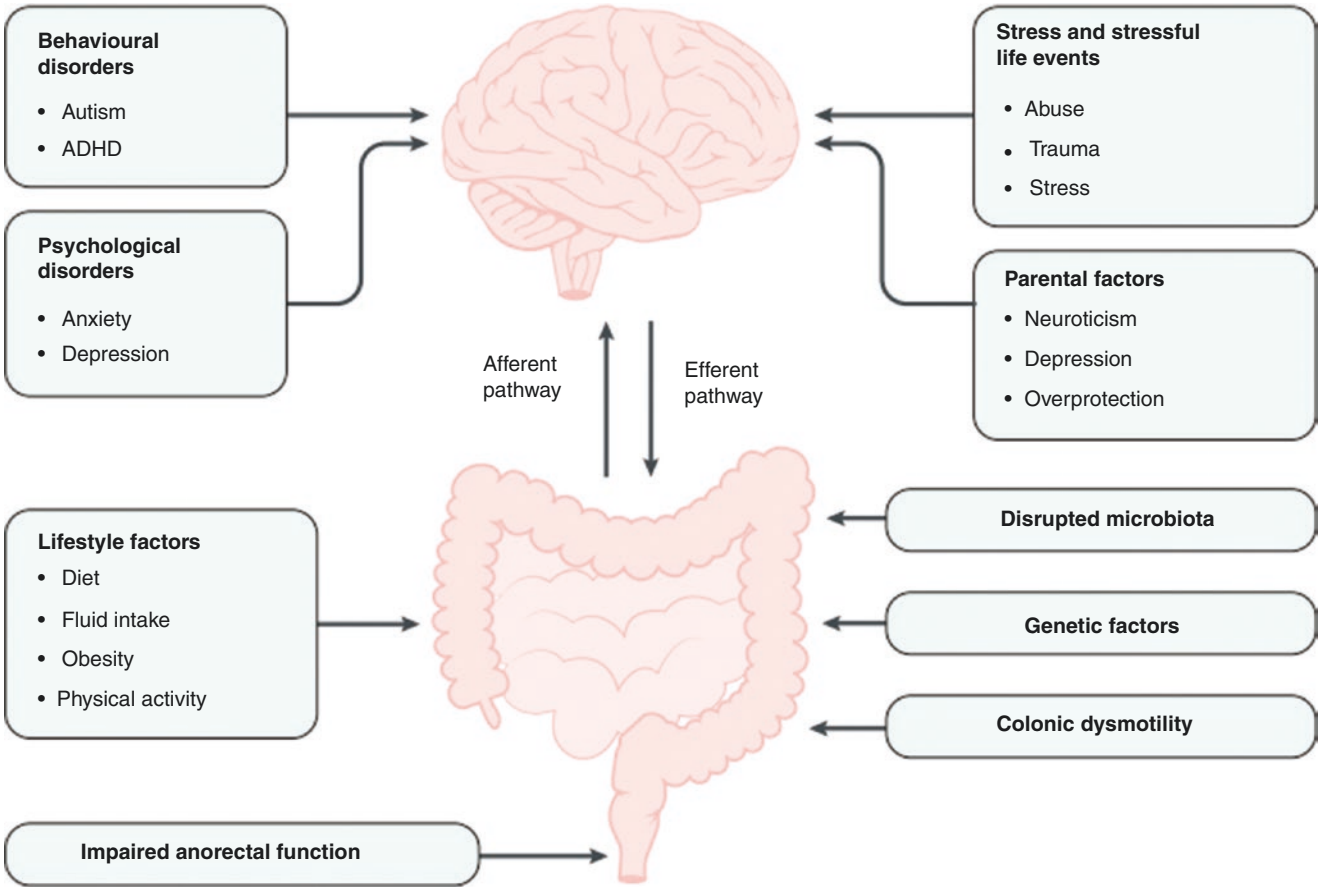

Fig. 21.1 An overview of the multifactorial pathophysiology of functional constipation. Psychological and behavioral factors are thought to affect the brain-gut axis both via efferent and afferent pathways. (Reused with permission from Vriesman et al. [87]). *ADHD* attention-deficit/hyperactivity disorder

stipation as different entities, evidence is accumulating that suggests that the two are part of a disease continuum. In this continuum, diagnosing one or the other may not only be a challenge, but the patient may actually move between diagnoses over time, switching from IBS to functional constipation or vice versa [99, 100]. In the majority of children who struggle with fecal incontinence, the incontinence is secondary to constipation. However, in approximately 5–10% of the children (mainly boys), fecal incontinence may occur without other symptoms of constipation. According to the Rome IV criteria, these children should be diagnosed with non-retentive fecal incontinence and should be treated accordingly. Part 2 of this chapter will discuss this separate clinical entity in detail. Poor appetite, nausea, vomiting, and abdominal bloating are other important features that need to be elicited during history taking. Lower urinary tract symptoms including urinary incontinence are also seen in some children, and refractory vulvovaginitis is a known feature, especially in prepubertal girls [101–104].

Past medical history, specially concentrating on factors that may cause constipation, such as medications or previous surgical corrections of anorectal malformations and Hirschsprung's disease, is also an important part in the evaluation [105, 106]. Dietary history particularly concentrating

Box 1 Alarm Symptoms of Constipation

Acute symptoms

- Delayed passage of meconium (first meconium after 48 hours of life)
- Fever, bilious vomiting, or diarrhea in a young infant, especially if there are other signs or risk factors for Hirschsprung's disease (e.g., Trisomy 21)
- Rectal bleeding (unless attributable to an anal fissure)
- Severe abdominal distension

Chronic symptoms and signs

- Very early-onset constipation (<1 month old)
- "Ribbon" stools (very narrow in diameter)
- Failure to thrive
- Extraintestinal symptoms (especially neurologic deficits)
- Physical findings suggestive of anorectal disorder, sacral spine abnormality, or sexual abuse (e.g., anteriorly displaced anus, scars, extreme fear of anal exam).
- Family history of Hirschsprung's disease, celiac disease, or hypothyroidism

on fiber content is an integral part as underconsumption of fiber may lead to constipation [36, 107–109]. When symptoms arise after introduction of cow's milk to the infant's diet, cow's milk allergy may play a role [42].

Psychological abnormalities also need to be uncovered as there is an association between children with functional constipation and behavioral problems [74–77]. In addition, stressful life events such as physical, sexual, and emotional maltreatment need to be evaluated carefully in children with constipation as these factors are known to predispose to the development of constipation [14]. Finally, consequences of constipation should be discussed. Studies have shown that children with functional constipation have a reduced health-related quality of life [46]. Children with constipation, with or without fecal incontinence, may often have to deal with feelings of shame, peer rejection, and bullying. Creating a comfortable place for them to defecate, like the teacher's bathroom in school, may contribute to adequate treatment and resolution of symptoms, as well as increasing their quality of life.

Physical Examination

Physical examination should start with assessment of growth. Faltering growth and short stature are alarm features of organic causes (endocrine, metabolic, etc.) of constipation.

Abdominal examination may reveal the presence of abdominal distension and past surgical scars from abdominal surgery. Palpable fecal masses in the lower abdomen indicate increased fecal loading, and this may be present in about 50% of children with constipation [110]. Gaseous distension is more in favor of constipation-predominant irritable bowel syndrome ("IBS-C").

Perianal examination may reveal abnormal position of the anus [111]. Fecal smears and perianal excoriation of skin indicate presence of fecal incontinence. Fissures and anal tags can also be seen in children with chronic constipation who pass hard stools, but may also indicate sexual abuse. A patulous anus (loose, open) is the result of a loss of tone of sphincter muscles due to damage or dysfunction of the muscles, or disturbances in their innervation, and can be associated with fecal incontinence [112, 113].

Digital rectal examination is not necessary in children whom already fulfill Rome IV criteria for a diagnosis of functional constipation [95]. However, if the diagnosis is unclear, if there is doubt between the diagnosis of non-retentive fecal incontinence and functional constipation, or if conventional treatment fails, digital rectal examination is indicated. During the examination, one should assess resting tone, squeeze pressure of the sphincter complex, and rectal fecal loading. Findings suggestive of Hirschsprung's disease are a tight anal canal with an empty ampulla. A digital rectal examination in patients with Hirschsprung's disease may temporarily relieve the obstruction and therefore result in an explosive release of gas and stool (blast sign or squirt sign). Findings suggestive of functional constipation are an enlarged rectum filled with (hard) stool. However, it is important to note that a lack of stool in the rectum does not exclude the diagnosis of functional constipation.

In addition to physical examination focused on the gastrointestinal tract, their neurological, cognitive, and social development should also be assessed, and dysmorphic features should be noted. This is necessary to identify children with syndromes, neurological disorders, or behavioral disorders mentioned earlier who are at risk to develop constipation [76, 114]. Neurological examination should concentrate specially on features of spina bifida, motor and sensory deficits in the lower limbs, and perianal sensory testing. One should evaluate perianal sensory loss and absence of anal wink.

Investigations

Constipation is a clinical diagnosis. With the use of current diagnostic criteria, additional testing to diagnose and successfully treat children is not necessary in most children with defecation disorders. However, if the diagnosis is still uncertain, if there is a suspicion for an organic cause of constipation, or if children do not respond to conventional medical treatment, focused additional investigations are indicated. The most commonly used additional tests include laboratory tests, imaging tests, and functional tests.

Laboratory Tests

At initial presentation, laboratory testing should only be performed in the presence of alarm signs suggestive of a metabolic cause of constipation. The additional value of routine testing of celiac disease or hypothyroidism has not been demonstrated [115–117]. Testing may include a complete blood count, biochemical profile, thyroid function, and celiac screening. As the association between cow's milk allergy and constipation remains controversial, testing for cow's milk allergy is not recommended, but a 2- to 4-week trial of avoidance of cow's milk may be considered [95].

Radiological Tests

One of the main reasons to perform imaging studies is to differentiate children with functional constipation with overflow incontinence from those with non-retentive fecal incontinence [95]. In addition, imaging is more often used in children who are not able to adequately report on their bowel symptoms, as may be the case in children with autism spectrum disorder [118].

Ultrasonography has been used to assess the degree of fecal retention in the rectum. Using a transabdominal approach, several studies have measured the rectal diameter to determine fecal loading in the rectum using different methods, and have

shown that children with chronic constipation have a larger rectal diameter compared to controls [119–121]. The results are promising, and the wide availability and noninvasive nature of the test makes it an ideal investigation. However, methods need to be standardized, and larger studies evaluating the reliability of measurements with blinding of the examiners are needed before routine use of ultrasonography in the assessment of children with constipation is recommended.

Plain abdominal X-rays are still often used to demonstrate fecal loading in the colon and rectum in order to diagnose constipation, and several scoring systems have been proposed to assess the degree of fecal loading [122, 123]. However, sensitivity and specificity of these scores are variable, and the inter- and intraobserver reliability is poor [124]. Moreover, studies have shown that the use of abdominal X-rays as tool to diagnose constipation may even be harmful. A large multicenter retrospective study found that children diagnosed with constipation who received an abdominal X-ray were twice as likely to return to the emergency department with a clinically significant alternate diagnosis like acute appendicitis [125]. A recent study showed that education on the unreliability of abdominal X-rays in the emergency department lowered the percentage of abdominal X-rays performed on healthy patients discharged home with a diagnosis of constipation while keeping the number of important return visits to the ED low [126]. Based on the current evidence, abdominal radiography is rarely justified when assessing constipation in children and children presenting with abdominal pain [95, 127].

Colonic transit time is usually assessed by using radiological methods. It generally gives an idea of propulsive function of the colon and may help to identify segments with abnormal motility. It is usually conducted using radiopaque markers or scintigraphic methods. In marker studies, radiopaque markers are ingested with a meal or swallowed as a capsule, and abdominal X-rays are obtained to count the number of markers in different segments of the colon. In scintigraphy, the patient is given a meal containing a radioisotope, and multiple images are taken using a gamma camera to assess the radioisotope count in each region. A delayed colonic transit time can indicate slow-transit constipation but may also result from withholding behavior in children [128, 129].

The use of barium (contrast) enema as an initial diagnostic tool for the evaluation of children with constipation is not recommended [95]. Contrast enemas are sometimes used in the evaluation for Hirschsprung's disease or to plan surgical intervention after diagnosis. However, rectal biopsy or anorectal manometry is more reliable in diagnosing Hirschsprung's disease, and the radiographic location of the transition zone does not always correlate accurately with the level of aganglionosis [130, 131].

Although the literature on the additional value of defecography in children with constipation is limited, the test is sometimes used to evaluate children with intractable constipation. Defecography is a dynamic radiologic test used to visualize anorectal function at rest and during voluntary defecation. It can be used to identify children with pelvic floor dyssynergia and structural abnormalities, including intrarectal intussusception, rectocele, or rectal prolapse [132]. A recent study examining the value of fluoroscopic defecography in 51 children with prolonged colonic transit times found abnormal defecography results in 27 patients (52.9%) [133]. Structural abnormalities were the most common abnormal finding (n = 15), followed by pelvic floor dyssynergia (n = 10) and a combination of the two (n = 2). However, the authors state that children with minor structural abnormalities continued medical treatment, and additional therapeutic consequences of defecography findings were not reported. Therefore the additional value of the test and its findings remains unclear.

Magnetic resonance imaging of the spinal cord should be considered in children with additional neurological abnormal findings to diagnose spina bifida occulta and terminal filum lipoma. In children without neurological abnormalities, the prevalence of lumbosacral spine abnormalities is low, and the significance of the abnormalities is unclear [134].

Functional Tests

Anorectal manometry allows assessment of the neuromuscular function of the anus and the rectum. With the use of a catheter in the anus and rectum, pressures in the anal canal can be measured and defecation dynamics can be visualized. The catheter contains a balloon placed in the rectum which can be inflated to mimic fecal rectal filling. By inflating the balloon, the nerves in the rectum should initiate a relaxation of the internal anal sphincter, resulting in a decrease in pressure. This rectoanal inhibitory reflex is absent in patients with Hirschsprung's disease, anal sphincter achalasia, after circular rectal myomectomy, or in some patients with (corrections of) anorectal malformations [135]. If manometry is performed awake, children can verbalize rectal sensation during different balloon inflations, and they can be asked to push and squeeze to evaluate anal sphincter function [135]. In case the child contracts instead of relaxes the anal sphincter, when attempting to expel the inflated balloon, a diagnosis of pelvic floor dyssynergia is made. However, because of the unnatural lateral position and setting (with presence of medical personnel), the results of this maneuver may be falsely positive [135]. Anorectal manometry testing may be considered in children with a low suspicion for Hirschsprung's disease in order to exclude Hirschsprung's disease (when there is a high suspicion, a rectal biopsy is indicated), or to evaluate for anal achalasia

or pelvic floor dyssynergia [135, 136]. However, the significance of these last two findings remains controversial as children with anal achalasia have only been minimally described in the literature and therapeutic modalities to treat pelvic floor dyssynergia, including biofeedback, have not proven their advantage over conventional treatment [63, 137, 138]. Next to conventional anorectal manometry testing, a new high-resolution catheter with 256 sensors providing a three-dimensional visualization of the anal canal has been developed. The topographic pressure measurements may demonstrate longitudinal and radial asymmetry of the anal canal and may allow for a better visualization of anatomical impaired anorectal function, as well as better understanding of the mechanisms of fecal continence in children [139]. Its usefulness in daily practice and impact on treatment have yet to be determined.

Colonic manometry allows the measurement of pressure/peristaltic movements of multiple regions within the colon in real time and helps to discriminate between normal colonic physiology and colonic myopathies and neuropathies [21]. A number of colonic motor patterns have been identified, such as antegrade HAPCs, low-amplitude propagating sequences, non-propagating contractions, and retrograde propagating pressure waves [67]. Studies have suggested that colonic manometry findings may predict outcomes of surgical interventions, and that an abnormal colonic manometry may be predictive of surgery [135, 140–142].

Management

Effective management of constipation requires a multifaceted approach. A stepwise management protocol is shown in Fig. 21.2. The main steps include lifestyle modification, toilet training, use of laxatives and enemas, biofeedback/pelvic floor physiotherapy, nerve stimulation, and surgical interventions. However, it is important to realize that the data in the pediatric literature to support evidence-based use of treatment strategies are limited, especially regarding old laxatives such as lactulose and bisacodyl. Therefore, the management mostly depends on individual experiences.

Lifestyle Modifications

As stated earlier, constipation is associated with psychological stress related to home, school, and society [18, 31, 48, 75]. These factors need to be addressed during consultation. Children with psychological stress need to be identified and coping mechanisms need to be taught. Home- and school-related punishment is another factor that is known to predispose children to develop constipation which can easily be avoided [28].

Although the prevalence of constipation is associated with a low intake of fiber, a high-fiber diet has not shown to relieve constipation [107, 143, 144]. Current treatment guidelines recommend a diet with a normal fiber intake (around 5g + the age of the child in years) [95]. Similarly, increase in the consumption of water (aside from the extra fluid necessary for laxative dissolvent) has also not shown to be beneficial in the treatment of constipation [145].

Other interventions with respect to previously described risk factors for constipation like decreased physical activity and frequent consumption of fried food have not yet been studied. However, general advice for a healthy lifestyle is never inappropriate and may be beneficial for the child's bowel habits. Following on the hypothesis on the effect of the microbiome on children with constipation, pre-, pro-, and synbiotics have become of interest as treatment modalities. However, so far current evidence does not support the use of these compounds as a single or additional therapy for treatment of functional constipation in children [146, 147].

Toilet Training and Behavioral Therapy

Stool withholding plays a crucial role in the development of constipation in young children. Children with constipation need to relearn how to properly pass stools in the toilet. As the first step, a negative attitude regarding stools needs to be eliminated. This facilitates and prepares the child's mentally to pass stools in the toilet or potty. The child is encouraged to use the toilet whenever he or she feels an urge and regularly after each meal as the postprandial gastrocolic reflex facilitates generation of propagating contractions which help to evacuate stools. The proper seating method (upright posture) to bring the anorectum in the correct angle to facilitate the passage of stools needs to be taught. Proper positioning of legs and relaxing the pelvic floor and anal sphincters should also be explained. Once the child masters these techniques, it is necessary to teach proper straining methods. This process needs to be a regular practice and may be encouraged with a reward system [148]. Evidence indicates that adding behavioral therapy to conventional laxatives has benefits in treating children with constipation [149, 150]. It is obvious that behavioral therapy alone cannot cure constipation. However, given the importance of stool-withholding behavior (especially in infants and younger children), toilet training and behavioral modifications are mandatory parts in the day-to-day clinical management of these children.

Subsequently, parents should be educated on the effects of their thoughts, attitudes, and behavior toward the child with constipation and fecal incontinence. Studies have shown that parental child-rearing attitudes are associated with defecation

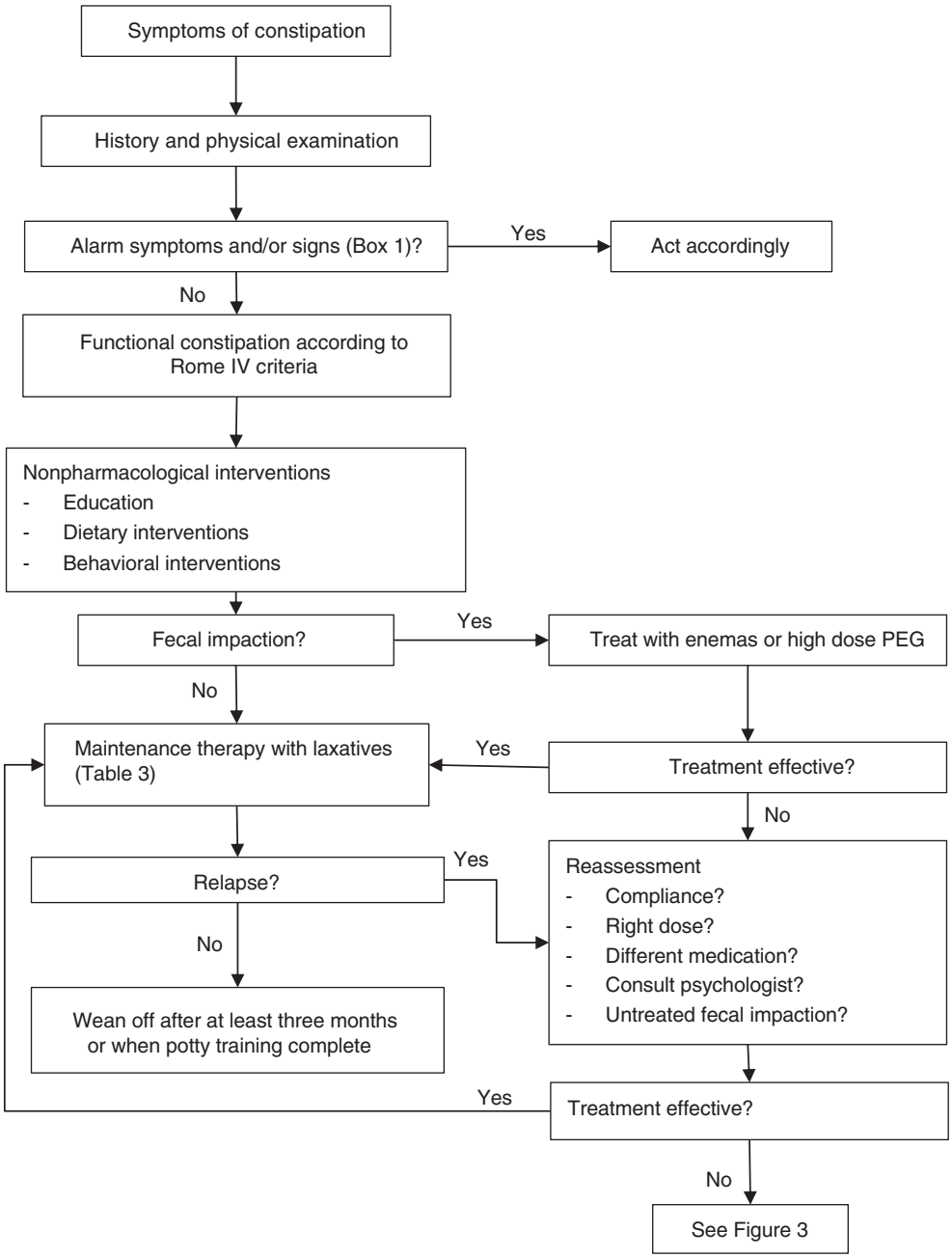

Fig. 21.2 Algorithm for initial presentation of symptoms of constipation. A suggested stepwise approach for the evaluation and treatment. (Modified with permission from Vriesman et al. [87]) *PEG* polyethylene glycol

and fecal incontinence frequency [80]. High levels of frustration or irritability toward the child have a negative effect on symptoms of fecal incontinence. Parents should be made aware that the episodes of fecal incontinence are not the child's fault and the child is not purposely losing stools. By avoiding blame and praising positive behavior, education on parental influence may enhance treatment [79, 151]. Moreover, in order to assure treatment adherence, parents should be educated on two main reasons for children with constipation having watery stools, namely "overflow diarrhea" and regular diarrhea. Overflow diarrhea is a result of severe constipation, and these are soft stools which pass around a solid, obstructing, fecal mass and can result in overflow incontinence. Regular diarrhea could be a consequence of too high laxative dose or may be caused by gastroenteritis.

Pharmacological Treatment

In addition to education, keeping a bowel diary, and starting toilet training, pharmacological treatment is part of the first step in treating children with constipation [95]. Pharmacological treatment is based on two cornerstones: disimpaction of the rectum and maintenance therapy.

Fecal Disimpaction of the Rectum

Disimpaction of the rectum is indicated whenever a child diagnosed with constipation presents with fecal impaction defined as: "a hard mass in the lower abdomen identified on physical examination or a dilated rectum filled with a large amount of stool on rectal examination or excessive stool in the distal colon on abdominal radiography" [95, 152, 153]. After evacuation of the fecal mass, children are more likely to respond to maintenance therapy [154]. Disimpaction can be achieved with high doses of oral laxatives or with enemas containing stimulant ingredients such as sodium phosphate or sodium docusate. In severe cases, or when the child refuses oral and rectal medications, manual disimpaction under general anesthesia is indicated. Studies comparing disimpaction with oral laxatives with disimpaction with enemas found both routes to be effective but found some benefit for the use of rectal enemas. Children using rectal enemas experienced a faster relief of symptoms, and high doses of polyethylene glycol (PEG; 1.5 g/kg/day) caused more fecal incontinence [152, 153]. However, despite therapeutic advantages of rectal treatment, it is imperative to realize the invasive nature of rectal therapy. This is especially important to keep in mind when the child has perianal pain, anal fissures, or suffers from morbid fear of manipulations around the perianal region. Although rectal disimpaction may be uncomfortable for the child and parent, usually the idea is more freighting than the act itself, and a practical advantage is the predictability of the timing of the evacuation of the fecal mass. Current guidelines recommend a high dose of orally administered PEG based on the argument that PEG can be easily administered orally [95]. Based on prior experience and possible fear of the child, the physician should come up with a best plan for fecal impaction individualized for each patient. One randomized trial in Jordan compared the effectivity of PEG (1.5 g/kg/day) and lactulose (4–6 ml/kg/day) in treating fecal impaction and found a faster response is the lactulose group, indicating lactulose may also be an alternative treatment option for disimpaction.

Maintenance Therapy

Once disimpaction is achieved, it is imperative to initiate maintenance therapy. This facilitates passage of stools and prevents re-impaction. Table 21.4 shows the details of drugs that are currently used in the management of childhood constipation. First choice of maintenance treatment are osmotic laxatives such as PEG. If PEG is not available, lactulose is the laxative of choice. As additional or second-line treatment, milk of magnesia (magnesium peroxide), mineral oil (liquid paraffin), and stimulant laxatives (senna or bisacodyl) may be considered [95]. In children with constipation after repaired anorectal malformations, stimulant laxatives are the first choice of maintenance treatment as modification of stool consistency by an osmotic laxative may lead to fecal incontinence [155]. According to current guidelines, maintenance therapy should continue for at least 2 months [95]. Discontinuation of treatment may be considered when all symptoms of constipation symptoms are resolved for at least 1 month and treatment should be decreased gradually. When children are in the developmental stage of toilet training, medication should be continued until toilet training is achieved.

Osmotic Laxatives

Osmotic laxatives are the group of choice used in the maintenance phase. As a group, they exert an osmotic effect which helps to increase the water content in the colon and hence softening stools in a dose-related response while being only minimally systemic absorbed and with minimal adverse effects. Lactulose and PEG are the two most commonly used osmotic laxatives. Due to its ease of administration, its effectiveness, and good safety profile, PEG is the laxative of first choice. Multiple studies have demonstrated the superiority of PEG over placebo, lactulose, mineral oil, and milk of magnesia (magnesium hydroxide) [156–162]. However, the results of these studies ought to be interpreted with caution due to quality and methodological concerns, as well as clinical heterogeneity, and short follow-up. Two types of PEG are available (PEG 3350 and PEG 4000) with or without addition of electrolytes [163]. The numbers after PEG correspond with the molecular weight of the drug molecule and the addition of electrolytes is thought to minimize the risk of electrolyte imbalances. A randomized controlled trial could not prove noninferiority of PEG 3350 with electrolytes compared to PEG 4000, but concluded that both treatments are likely to have similar long-term efficacy and safety [164]. Overall fluid intake may be a confounding factor in the effectivity of PEG treatment, where poor fluid intake may decrease effectivity [165]. An adult study found that PEG 4000 with orange flavor (Forlax) has at better taste than PEG 3350 with electrolytes (Movicolon) [166]. Questions on the safety of long-term use of PEG in children have been raised due to reports of neuropsychiatric events and the use of PEG 3350, but the United States Food and Drug Administration (FDA) states that based on current available data no action is required [167]. As previously mentioned, co-occurrence of behavioral problems and functional constipation has been extensively described and may explain the reports of neuropsychiatric events [74–77]. In addition, a case-control study found no sustained elevated blood levels of neurotoxins (ethylene glycol and diethylene glycol) in children who used PEG 3350 daily compared with healthy controls [168]. It is important to discuss any parental concerns, misperceptions, and satisfaction on the use of laxative as parental perceptions have been shown to be associated with PEG treatment adherence, which overall is found to be low (37%) [169] (Fig. 21.3).

Table 21.4 Pharmacological agents for childhood functional constipation

Drug	Evidence	Side effects	Dose
Osmotic laxatives			
Polyethylene glycol (PEG)	Improves consistency and frequency of stools, and straining	Diarrhea and abdominal distention	Maintenance: 0.3–0.8 g/kg per day; disimpaction: 1–1.5 g/kg per day (for maximum of 7 days)
Lactulose	Improvement of symptoms of mild to moderate constipation; safe to use in young children	Abdominal gas, bloating, and cramping	7 months–18 years: 1–2 g/kg per day in 1–2 doses
Milk of magnesia (magnesium hydroxide)	Evidence of efficacy is poor	Diarrhea	2–5 years: 0.4–1.2 g per day; 6–11 years: 1.2–2.4 g per day; 12–18 years: 2.4–4.8 g per day
Lubricants			
Mineral oil (liquid paraffin)	Evidence on efficacy is of poor quality	Skin irritation and reduced absorption of fat-soluble vitamins; risk of lipid pneumonitis with aspiration	1–3 ml/kg per day (maximum 90 ml per day)
Stimulant laxatives			
Bisacodyl (diphenylmethane)	Improvement of symptoms	Oral: diarrhea and abdominal pain Rectal: abdominal pain and anal discomfort	Oral or rectal: 3–10 years: 5 mg per day in 1 dose before bedtime; >10 years: 5–10 mg per day in 1 dose before bedtime
Sodium picosulfate (diphenylmethane)	Improvement of symptoms	Diarrhea and abdominal pain	4–5 years: 3 mg; >6 years: 4–6 mg per day in 1 dose
Senna (anthraquinone)	Clinical experience suggests effectiveness, but no large randomized trials have been performed	Diarrhea and abdominal pain; in young children risk of dermatitis	2–6 years: 2.5–5 mg/day in 1–2 doses; 6–12 years: 7.5–10 mg/day in 1–2 doses; >12 years: 15–20 mg/day in 1–2 doses
Rectal enemas for fecal disimpaction			
Sodium docusate	Evidence supports effect on fecal impaction	Abdominal pain and anal discomfort	<6 years: 60 ml; >6 years: 120 ml
Sodium phosphate	Evidence supports beneficial effect on fecal impaction	Water and electrolyte disturbances, especially in young children; do not use when suspicious of Hirschsprung disease	2.5 ml/kg (maximum of 133 ml per dose)
Sodium lauryl sulfoacetate	Evidence supports effect on fecal impaction	Abdominal pain and anal discomfort	1 month–1 year: 2.5 ml/dose; >1 year: 5 ml/dose

Lubricant Laxatives

Although there are no placebo-controlled trials involving lubricant laxatives, two trials comparing mineral oil with lactulose have noted statistically significant better response rates with mineral oil [170, 171]. When comparing mineral oil with PEG, PEG was found to be superior due to higher response rates and/or fewer adverse events [161, 172]. It is important to note that due to the risk of life-threatening lipoid pneumonia, mineral oil is not recommended for young infants and children with swallowing difficulties, especially those who are neurologically impaired [173].

Stimulant Laxatives

Bisacodyl and senna stimulate peristaltic movements and enhance fecal evacuation. Bisacodyl can be taken orally and as suppository. Senna can only be taken orally. There are no good quality trials which evaluate stimulant laxatives in childhood constipation. Studies comparing senna with lactulose and mineral oil demonstrated that children using senna had a poorer response [174, 175]. A recent study including long-term retrospective data of 164 children showed that bisacodyl is effective and well tolerated in children with functional constipation refractory to conventional treatment [176]. Another study evaluating the effect of bisacodyl on colonic motility found that in 93% of children with intractable constipation, bisacodyl induced HAPCs [69]. Despite the lack of well-designed trials, these drugs are commonly used in day-to-day clinical practice, often combined with osmotic laxatives.

New Drugs

Due to the fact that many children treated for functional constipation remain symptomatic after 6–12 months of treatment [177] and 25% of children remain symptomatic into adulthood [178], new drugs have been studied. Some of these new therapies seem promising. However, difficulties arise in extrapolating adult data to the pediatric population

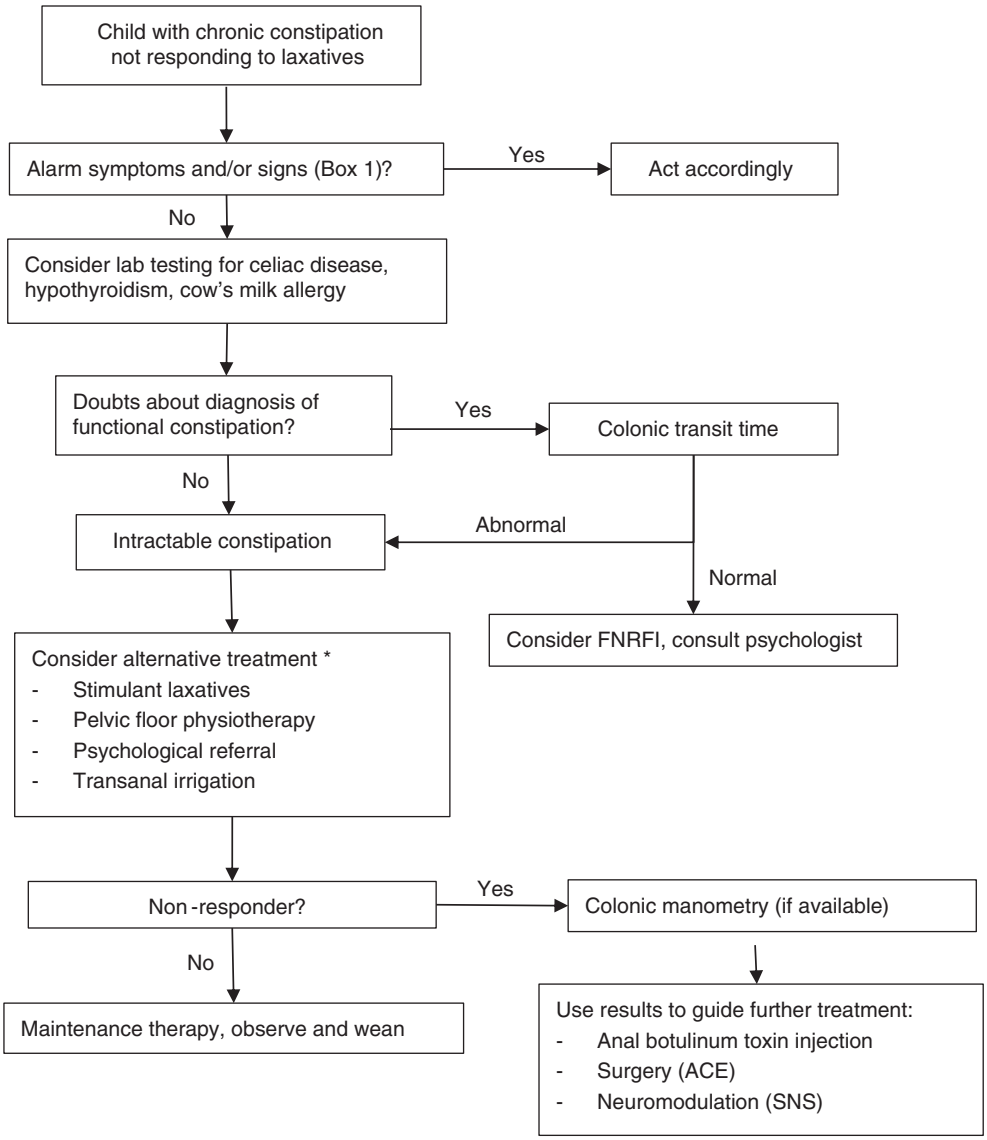

Fig. 21.3 Algorithm for children with functional constipation not responsive to laxatives. A suggested stepwise approach for the evaluation and management of children with chronic functional constipation who do not respond to conventional strategies. *FNRFI* Functional non-retentive fecal incontinence, *ACE* antegrade continence enema, *SNS* sacral nerve stimulation. *Assess compliance and dosage, use of other medication, and combination therapy. (Modified with permission from Vriesman et al. [87])

due to differences in pathophysiology, pharmacodynamics, pharmacokinetics, and importance of different outcome measures in the two populations. Lack of high-quality placebo-controlled randomized controlled trials and high heterogeneity make it challenging to assess the benefits of these novel treatments. In addition, the relatively high costs of these novel agents have to be taken into consideration. To ensure the setup of comparable, high-quality studies, recommendations for pharmacological clinical trial for children with constipation have been published [10].

Prosecretory Agents

Prosecretory agents modulate epithelial channels in the gut, thereby promoting the intestinal secretion of fluids and enhancing stool volume, resulting in an improvement in gastrointestinal transit [179]. Currently available agents include lubiprostone, linaclotide, and plecanatide.

Lubiprostone

Lubiprostone is a locally acting chloride channel activator specific to the gastrointestinal tract. It promotes intestinal secretion of chloride ions and fluid and gastrointestinal motility and has a good track record in treating adults with constipation [180, 181]. In an open-label pediatric trial, lubiprostone was shown to be safe and effective in improving spontaneous bowel movements, reducing episodes of FI, and reducing many other symptoms of constipation [182]. However, early findings of a double-blind, placebo-controlled, multicenter trial studying lubiprostone in children with functional constipation found no significant difference between groups in the primary endpoint (increase of >1 bowel movement above baseline and >3 weekly bowel movements for at least 9 weeks, with responses for 3 of the final 4 treatment weeks) [183]. Some beneficial effects on secondary endpoints (spontaneous bowel movement fre-

quency and painfulness, straining, and stool consistency) suggest that lubiprostone may still provide clinically relevant benefits.

Linaclotide

Linaclotide promotes intestinal fluid secretion by activating the guanylate cyclase C-receptor. It is approved for the treatment of adults with functional constipation, but pediatric data remain scarce [184]. Preliminary results from a multicenter, randomized, double-blind, placebo-controlled, parallel-group, safety and efficacy dose-finding study in children with constipation-predominant irritable bowel syndrome showed that linaclotide was safe and well tolerated at daily doses up to 145 and 290μg in patients aged 7–11 and 12–17 years, respectively. A trend toward greater efficacy was observed with increasing doses for the primary endpoint and secondary endpoints related to bowel habits [185]. Results from a retrospective study in a combined population of children with functional constipation (68%) and irritable bowel syndrome (32%) found the majority of patients with functional constipation experienced an improvement of symptoms, but side effects, such as diarrhea and abdominal pain, were common and nearly a third of patients had stopped taking linaclotide within the first months of treatment mostly because of these side effects [186]. Future research is warranted before linaclotide should be used in children with functional constipation.

Plecanatide

Plecanatide is a new guanylate cyclase C-receptor agonist approved for the treatment of adults with constipation in the United States. No data are available on the use of plecanatide in children.

Serotonergic Agents

Serotonin is a central and enteric neurotransmitter that increases the release of enteric acetylcholine by binding to 5-hydroxytryptamine 4 receptors in the gut, which results in an increased secretion and enhances gut motility [187]. Several selective serotonin agonists have been developed to treat functional constipation including prucalopride, velusetrag, and naronapride. So far, only prucalopride is FDA approved to use in adults with constipation. Prucalopride has a clear gastroprokinetic activity and selective and high affinity for 5-HT4 receptor agonists that stimulate lower gastrointestinal motility. Although the results of an open-label trial in children looked promising [188], a large multicenter pediatric randomized controlled trial showed that prucalopride was not more effective in increasing stool frequency or decreasing fecal incontinence frequency compared to placebo and its use is therefore not recommended [189]. The lack of benefit of prucalopride in children compared to adults is likely the result of the high prevalence of stool withholding in children as in a study in adults with rectal evaluation disorders prucalopride was not superior to PEG [190]. This indicates that a prokinetic drug is not likely to affect rectal evacuation, resulting in the inability to overcome stool withholding in children.

Biofeedback and Pelvic Floor Physiotherapy

Anorectal function can be modified with the use of biofeedback or pelvic floor physiotherapy [191]. Biofeedback training entails teaching children how to coordinate muscle relaxation with the use of anorectal monitoring instruments in order to amplify physiological processes and make physiological information accessible to the child's consciousness [63]. Pelvic floor physiotherapy consists of exercises, practicing a stabilized posture on the toilet, teaching effective straining to defecate, increasing awareness of sensations, and exercising adequate pelvic floor muscle functions [192]. The purpose of both treatments is to improve anorectal function and sensation [193]. Several studies have shown efficacy of biofeedback in correcting defecation dynamics, but well-conducted large randomized controlled trials failed to find additional clinical benefits of biofeedback in children with functional constipation [63, 194]. Moreover, normalization of defecation dynamics was not associated with treatment success [195]. In contrast, pelvic floor physiotherapy has shown to be more effective than standard medical care in the treatment of children with constipation [192]. Differences in outcomes between both therapies may be explained by the differences in providers; biofeedback is usually provided by a medical care provider, whereas pelvic floor physiotherapy is provided by a physiotherapist. The broader approach of pelvic floor physiotherapy focused on children's awareness of all their bodily sensations, while learning to recognize and resolve abnormal functions, or to prevent fecal incontinence, also may explain the difference in effect.

Trans-anal Irrigation

During trans-anal irrigation, a catheter or a cone is inserted in the anus through which a large volume of water is infused to clear fecal contents in the rectum and parts of the colon. It is an accepted treatment in children and adults with bowel disorders. Recent studies using trans-anal irrigation in children with bowel dysfunction have reported high success rates, both in clinical outcomes and in effect on quality of life [196–199]. Experience in children with functional constipation is limited to a few cohort studies reporting positive effects [196, 197, 200]. Even though no randomized controlled trials exist, due to its conservative nature and reported effectivity, it is recommended to try trans-anal irrigation before considering surgical interventions in children with intractable constipation [201]. A recent multicenter evaluation on adherence to trans-anal irrigation showed that performing at least one irrigation under a nurse's supervision, discontinuing constipation treatment, and advising to irrigate daily was related to continuation of trans-anal irrigation [202].

Neuromodulation

Although its working mechanism is far from clear, neuromodulation has been identified as a successful therapeutic modality for elimination disorders and is gaining increasing interest [203]. There are both invasive and noninvasive modalities available: implantable or transcutaneous sacral nerve stimulators, percutaneous or transcutaneous posterior tibial nerve stimulators, or interferential therapy.

Sacral nerve stimulation (also known as sacral neuromodulation) is currently the most established form of neuromodulation in children with defecation disorders. It involves electrical stimulation of the sacral nerve root via an electrode surgically placed in the sacral foramen. This electrode is connected to a pulse generator that is implanted within the subcutaneous fat of the buttock, often after an initial trial period during which the pulse generator remains external [204]. The electrical pulses are thought to modulate anorectal function at the pelvic afferent or central level [205]. Multiple studies have shown sacral nerve stimulation to be effective in treating children with constipation [203, 206]. However, the results of these studies must be interpreted with caution as they only contain small sample sizes and none of them were sham-controlled. In addition, a recent review on the effectiveness of sham nerve stimulation in adults showed sham stimulation is associated with both clinically and statistically meaningful improvements [207]. Next to this, reported complication rates of sacral nerve stimulation are high (18–55%) [203]. A proportion of children require additional surgery for lead revision, device removal, or device replacement, most often performed because of lead displacement or malfunction, local pain or numbness, and local infection [206, 208]. Therefore, noninvasive techniques have become more of interest as treatment modalities, and before using sacral nerve stimulation on a regular basis, a well-designed sham-controlled trial with a larger patient sample and a long-term follow-up is needed to evaluate its effectiveness and identify risk factors for complications.

Transcutaneous stimulation may involve stimulation with current delivered as spikes aiming to produce muscle contraction (functional electrical stimulation) or nerve stimulation (transcutaneous nerve stimulation), or with alternating current to intersect deeper within the tissue (interferential stimulation) [209]. Transcutaneous nerve stimulation delivered of the sacral nerve has only been studied in children in one pilot study with small patient sample ($n = 14$) with both lower urinary tract dysfunction with constipation and showed improvement according to Rome III criteria [210]. Transabdominal (interferential) electrical stimulation involves the generation of two sinusoidal currents that cross within the body with the use of four electrode pads applied on the skin of the abdomen and lower back [211]. Using this technique, Clarke et al. showed in eight children with slow-transit constipation a generation of significantly higher frequency of high amplitude and total propagatory sequences [212]. In addition, the same research group showed, in a randomized sham-controlled trial in children with slow-transit constipation, that transabdominal electrical stimulation (delivered by a physiotherapist (three 20-min sessions a week)) substantially improved colonic transit time, but no data on clinical symptoms were reported [213]. They also found an improvement in quality of life; however, the basal quality-of-life scores in the two groups were not similar, thus precluding any valuable conclusion [214]. Long-term follow-up of 30 children all recruited during previous mentioned studies showed that over 62% of subjects perceived improvement of at least one symptom (defecation, soiling, abdominal pain) lasting over 2 years in 27% to 37% of subjects [215]. Further studies are needed to confirm these findings and to determine the use of transcutaneous sacral nerve stimulation and interferential electrical stimulation in clinical practice.

Posterior tibial nerve stimulation entails electrical stimulation of the posterior tibial nerve at the level of the medial malleolus with the use of a needle (percutaneous) or self-adhesive electrode (transcutaneous) and is derived from acupuncture. Stimulation of the tibial nerve is thought to modulate bowel function through indirect stimulation of similar sacral nerves as stimulated during sacral nerve stimulation. Only one small study including eight children with fecal and urinary incontinence has investigated the effect of posterior tibial nerve stimulation and found improvement in symptoms [216]. Until more evidence on the effectiveness of this treatment becomes available, posterior tibial nerve stimulation is reserved for the academic setting.

Surgical Interventions

When all conventional therapies fail and symptoms of constipation continue to greatly disrupt quality of life, surgical treatment may offer benefit [95]. Currently used surgical interventions include procedures focused on the anal sphincter (anal botulinum toxin injections, anal dilation, and anal myectomy) and procedures focused on the colon (antegrade continence enemas, colostomy, and colonic resection). To date, no clinical guidelines on the surgical treatment of children with constipation exist and surgical decision-making differs widely among physicians [217]. Available evidence is mainly of low quality, contains only small heterogeneous patient samples, and there is a clear lack of qualitative outcomes assessors [218, 219]. In general, a step-up approach from least to most invasive procedure is followed.

Although only limited evidence is available, injection of botulinum toxin (single or multiple) into the anal sphincter seems to be safe and effective in children with functional constipation regardless of anal sphincter dynamics [220–222]. Botulinum toxin blocks the release of acetylcholine from neurons resulting in temporary chemical paralysis of muscle fibers, thereby relaxing anal sphincter muscles. It is speculated that disruption of the vicious cycle of stooling withholding may be achieved with botulinum toxin injec-

tion leading to functional recovery and normal stooling patterns. However, it is not uncommon to need repeat injection after 3–6 months. Temporary adverse effects frequently encountered include transient anal and abdominal pain and occurrence of fecal incontinence [223]. Because of the need for repeat injection and the requirement of general anesthesia, this is an expensive treatment. A recent randomized controlled trial evaluating the additional effect of anal botulinum toxin injection to standard bowel management treatment including a stimulant laxative (senna) in children with obstructed defecation did not find a significant effect of the injection on defecatory symptoms as measured by the Rintala score [224].

Anal myectomy and anal dilation have been largely abandoned by surgeons due to the risk of weakening the anal sphincter to the point of causing persistent fecal incontinence [217].

Common diagnostic workup before surgical colonic interventions often includes colonic motility testing [140, 142]. If segmental colonic dysmotility is present, it feels intuitive to remove or bypass the dysmotile colonic segment. However, even though this indeed has favorable outcomes [225], the dysmotility also seems to be potentially reversible and can improve after successful medical treatment [141, 226, 227]. The first procedure often considered in colonic surgery is a Malone appendicostomy or laparoscopic-assisted percutaneous cecostomy in order to allow the use of antegrade continence enemas. With the creation of an opening of the colon on the abdominal wall, the colon can be antegradely flushed. Effective cleansing agents include PEG solution and normal saline, combined with a stimulant laxative (bisacodyl or glycerin) if needed. Despite high success rates and good clinical outcomes, complications are common and include granulation tissues around stoma, leakage, and minor infections [218, 219]. Major complications such as fistulae, peritonitis, and stenosis of the stoma have been reported in a minority of patients [219]. Other surgical procedures such as sigmoid resection, colorectal resection, subtotal colectomy, and proctocolectomy with ileoanal anastomosis are only reserved for children with intractable constipation who failed to respond to all other therapeutic modalities [228, 229].

Part 2: Fecal Incontinence

Introduction and Epidemiology

Fecal incontinence is defined as passing stools in inappropriate places by a child whose development age is over 4 years [4]. Like constipation, fecal incontinence is a widespread problem, affecting up to 4.2% of children over 4 years of age [230]. It is more common among younger children, males, children with a positive family history and children from low socioeconomic strata and is associated with the occurrence of important life events such as the birth of a younger sibling and other psychological factors [230–236]. In addition, bullying, psychological stress, behavioral and upbringing problems, and poor social and school performances are commonly seen in children with fecal incontinence [51, 237].

Once organic causes are excluded, functional fecal incontinence may be the result of functional constipation or may occur on its own as functional non-retentive fecal incontinence (FNRFI). The vast majority of children with fecal incontinence have constipation-associated (retentive) fecal incontinence; only 5–10% of children can be diagnosed with FNRFI [231, 238, 239]. As the first already has been discussed in detail in the first part of this chapter, this section is devoted to FNRFI.

Functional Non-retentive Fecal Incontinence

Children with FNRFI pass normal stools in the toilet with a normal defecation pattern. They do not show features of constipation such as withholding behavior, pain, or difficulty in passing stools. However, they pass entire stools in inappropriate places for at least once a month. The Rome IV criteria to define FNRFI are given in Box 2.

A recent meta-analysis reported that the prevalence of FNRFI ranges from 0% to 1.8%, with a worldwide pooled prevalence of 0.4% (95% CI 0.2–0.7) [14]. The pathophysiological mechanism underlying FNRFI is still an enigma to pediatric gastroenterologists. Several studies have demonstrated normal colonic transit times (both segmental and total) in children with FNRFI [239, 240]. A study reporting data of colonic transit times in 161 children with functional defecation disorders showed that children with constipation-associated fecal incontinence were more likely to have significantly delayed total colonic transit compared to children with FNRFI [240]. Similarly, anorectal manometry and rectal barostat studies in children with FNRFI found that all parameters (rectal compliance, sensory thresholds) are within the normal range [59]. Many caregivers are convinced that fecal incontinence is the result of behavioral problems, and thus patients are often seen by a mental healthcare professional [232]. Indeed, children with FNRFI have significantly more behavioral problems than their healthy counterparts [241, 242]. However, most abnormalities in psychological parameters like anxiety or depression symptoms did not reach clinically significant levels and many of them disappeared after successful treatment. This suggests that in children with FNRFI, the fecal incontinence and behavioral abnormalities may be intertwined with each other and play an important role in each other's persistence [243]. Next to behavioral problems, urinary incontinence is commonly encountered in children with FNRFI indicating common physiological disturbances of

the bladder and the bowel [102, 232]. Bladder bowel dysfunction is an emerging new concept that describes concomitant malfunctioning of the lower urinary tract and the gastrointestinal tract [244, 245]. Treatment of urinary incontinence can positively affect FNRFI symptoms, and adequate treatment of FNRFI is often accompanied by a reduction in the number of urinary incontinence episodes [245, 246]. These findings suggest a combined, possibly neurodevelopmental, or behavioral disorder responsible for bladder and bowel dysfunction.

Box 2 Diagnostic Criteria for Non-retentive Fecal Incontinence [4]

At least a 1-month history of the following symptoms in a child with a developmental age older than 4 years:

1. Defecation into places inappropriate to the sociocultural context.
2. No evidence of fecal retention.
3. After appropriate medical evaluation, the fecal incontinence cannot be explained by another medical condition.

Clinical evaluation of children with defecation disorders has been discussed previously in the first part of this chapter. In addition to the described clinical evaluation, it needs to be stressed that digital rectal examination is important in children presenting with fecal incontinence only. It provides information on the presence of a fecal mass, anorectal sensation, and sphincter tone, and is essential to exclude functional constipation as cause of fecal incontinence [243]. Next to a detailed clinical evaluation, due to the high co-occurrence of behavioral disorders in children with FNRFI, it is recommended to screen for psychological difficulties with the use of validated questionnaires such as the Strengths and Difficulties Questionnaire or the Child Behavior Checklist [243, 247, 248].

Except for determining colonic transit time when the distinction between functional constipation and FNRFI is not apparent on clinical history and physical examination, additional investigations are not considered useful in the routine workup of children with fecal incontinence [243].

Management of children with FNRFI is challenging and often fraught by successes and failures [234]. Education and strict toilet training following an individualized behavioral routine and positive reinforcement are the four main cornerstones in management [243]. Rewarding should be focused on praising the scheduled toilet sits instead of periods without accidents, as most episodes of fecal incontinence occur involuntarily [249]. Conventional treatment modalities such as oral or rectal laxatives and biofeedback are not helpful [243, 250, 251]. Loperamide, an opiate receptor agonist, was found to be effective in a case report [252]. It is hypothesized that loperamide improves sphincter function and thereby prevents fecal incontinence. Further studies are needed to assess its efficacy and safety. When prescribing loperamide, careful supervision is required to prevent the development of constipation. Fecal incontinence in children with functional constipation with or without pelvic floor dyssynergia has been successfully treated with pelvic floor physiotherapy, or trans-anal irrigation, which may be also of value in children with FNRFI [196, 200, 253]. However, evidence for these treatments is children with FNRFI is lacking. A recent randomized controlled trial evaluating the additional value of transabdominal electrical stimulation to pelvic floor physiotherapy in 34 children with bladder bowel dysfunction found that in all children in the combined intervention group demonstrated improvement of daytime incontinence [254]. These findings indicate that pelvic floor physiotherapy and neuromodulation may be of value in the treatment of children with urinary and fecal incontinence. Although treatment is challenging and patients with FNRFI usually run a relapsing and remitting course, long-term follow-up demonstrates that in the majority (85%) of patients, symptoms have resolved by the age of 18 years [232].

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Hirschsprung's Disease and Intestinal Neuronal Dysplasias

22

Massimo Martinelli and Annamaria Staiano

Introduction

Hirschsprung's disease (HD) is a heterogesneous genetic disorder, resulting from an anomaly of the enteric nervous system of neural crest cells origin. HD is a congenital malformation characterized by the absence of parasympathetic intrinsic ganglion cells in the submucosal and myenteric plexus. It is the result of the premature arrest of the craniocaudal migration of vagal neural crest cells in the hindgut, between the fifth and twelfth week of gestation, to form the enteric nervous system and is therefore regarded as a neurocristopathy [1].

Epidemiology

HD occurs in approximately 1 of each 5000 live births and with a male predominance of 4:1. It is generally sporadic, although in 3–7% of cases a genetic transmission has been reported [2]. The risk for short segment disease is 5% in brothers and 1% in sisters of index cases; for long segment disease, the risk is 10%, regardless of sex [2].

Etiology

HD is characterized by the congenital absence of ganglion cells in the submucosal and myenteric plexuses in the distal bowel and variable proportion of the colon proximally. The embryonic disorder is a lack of the craniocaudal migration, differentiation, and maturation of neuroblasts from the neural crests, by the fifth to the twelfth week of gestation; the earlier the migration ceases, the longer the aganglionic segment will be. The aganglionic segment is permanently contracted, causing dilatation proximal to it [3]. HD may be classified according to the length of the aganglionic segment: the classic form (short segment 70–75% of cases) is limited to the rectum and sigmoid colon; the long segment, or subtotal colonic disease (10–15%), generally involves the bowel up to the splenic flexure; total colonic aganglionosis (TCA: 3–6%) may extend to involve a variable amount of the short bowel; and total intestinal aganglionosis is sometimes associated with intestinal malrotation or volvulus [2]. Ultrashort segment aganglionosis is considered a functional alteration, without any detectable histological finding. Although longer aganglionic segments tend to produce more dramatic symptoms, some patients with even short segment disease deteriorate rapidly [4].

Pathophysiology

The hallmark of diagnosis is the absence of ganglion cells from the myenteric and submucosal plexuses, as seen on a full-thickness or suction (mucosal-submucosal) biopsy of the rectum. Proximal contents fail to enter the unrelaxed, aganglionic segment. The lack of non-adrenergic-non-cholinergic inhibitory innervation is responsible for a tonic contraction of the affected segment, with absence of peristalsis and proximal dilation of the gut [5].

Morphologically, ganglionic cells are absent from the narrowed segment and for some distance (1–5 cm usually) into the dilated segment. The pattern of nerve fibers is abnormal also; they are hypertrophic with abundant, thickened bundles. Specific stains for acetylcholinesterase are used to highlight the abnormal morphology [6, 7].

In recent years, new insights in the pathophysiology of HD have been gained. It has also been suggested that abnormal expression of muscular neural cell adhesion molecule is likely to be associated with an arrest in the craniocaudal migration of neural cells to their most distal location [8]. Furthermore, the lack of nitric oxide (NO)-producing nerve

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fibers in the aganglionic intestine probably contributes to the inability of the smooth muscle to relax, thereby causing lack of peristalsis [9]. In addition, in the aganglionic segments, interstitial cells of Cajal are scarce and their network appears to be disrupted [10].

Genetics

HD occurs as an isolated trait in 70% of patients, is associated with chromosomal abnormality in 12% of cases, trisomy 21 being by far the most frequent (>90%). Additional congenital anomalies are found in 18% of cases, including gastrointestinal malformation, cleft palate, polydactyly, cardiac septal defects, and craniofacial anomalies. The higher rate of associated anomalies in familial cases than in isolated cases (39% vs. 21%) strongly suggests syndromes with Mendelian inheritance [2]. Isolated HD appears to be a multifactorial malformation with low, sex-dependent penetrance, variable expression according to the length of the aganglionic segment, and suggesting the involvement of one or more gene(s) with low penetrance [2]. These parameters must be considered for accurate evaluation of the recurrence risk in relatives. Segregation analyses suggested an oligogenic mode of inheritance in isolated HD. With a relative risk as high as 200, HD is an excellent model for the approach to common multifactorial diseases [11].

A large number of chromosomal anomalies have been described in HD patients. Free trisomy 21 (Down syndrome) is by far the most frequent, involving 2–10% of ascertained HD cases [12]. Syndromes associated with HD can be classified as: [1] pleiotropic neurocristopathies; syndromes with HD as a mandatory feature; and occasional association with recognizable syndromes. The neural crest is a transient and multipotent embryonic structure that gives rise to neuronal, endocrine and paraendocrine, craniofacial, conotruncal heart, and pigmentary tissues. Neurocristopathies encompass tumors, malformations, and single or multifocal abnormalities of tissues mentioned above in various combinations. Multiple endocrine neoplasia type 2 (MEN 2) and Waardenburg syndrome are the most frequent neurocristopathies associated with HD [13, 14].

Waarderburg syndrome (WS), an autosomal dominant condition, is by far the most frequent condition combining pigmentary anomalies and sensorineural deafness, resulting from the absence of melanocytes of the skin and the stria vascularis of the cochlea. The combination of HD with WS defines the WS4 type (Shah-Waardenburg syndrome). Indeed, homozygous mutations of the endothelin pathway and heterozygous SOX10 mutations have been identified in WS4 patients with CNS involvement including seizures, ataxia, and demyelinating peripheral and central neuropathies [15].

A wide spectrum of additional isolated anomalies has been described among HD cases with an incidence of sporadic types varying from 5% to 30% [2, 16]. No constant pattern is observed. These anomalies include distal limb, sensorineural, skin, gastrointestinal, central nervous system, genital, kidney, cardiac malformations, and facial dysmorphic features.

These data highlight the importance of a careful assessment by a clinician trained in dysmorphology for all newborns diagnosed with HD. Skeletal x-ray and cardiac and urogenital ecographic survey should be systematically performed. The observation of one additional anomaly to HD should prompt chromosomal studies.

Molecular Genetics

Several genes have been implicated in isolated HD, the two major ones being the proto-oncogene *RET* (*RET*) and the endothelin B receptor (*EDNRB*) [2].

The RET Signaling Pathway

The first observation was about an interstitial deletion of chromosome 10q11.2 in patients with TCA and mental retardation [17]. The proto-oncogene *RET*, identified as disease causing in MEN 2 and mapping in 10q11.2, was regarded as a good candidate gene owing to the concurrence of MEN 2A and HD in some families and the expression in neural crest-derived cells. Consequently, *RET* gene mutations were identified in HD patients [18]. Expression and penetrance of a *RET* mutation is variable and sex dependent within HD families (72% males and 51% females). Over 100 mutations have been identified including large deletions encompassing the *RET* gene, microdeletions and insertions, nonsense, missense, and splicing mutations [19–21]. Haploinsufficiency is the most likely mechanism for HD mutations. Biochemical studies showed variable consequences of some HD mutations (misfolding, failure to transport the protein to the cell surface, abolished biological activity).

Despite extensive mutation screening, a *RET* mutation is identified in only 50% of familial and 15-20% of sporadic HD case [22]. However, most families with few exceptions are compatible with linkage at the *RET* locus [23]. Mutations in RET ligand, like GDNF, GFRA1-4, NTN, persephin (*PSPN*), and artemin (ARTN), may occur, but are not sufficient to lead to HD.

The Endothelin Signaling Pathway

A susceptibility locus for HD in 13q22 was suggested for three main reasons: a significant lod score at 13q22 in a large

inbred Old Order Mennonite community with multiple cases of HD; de novo interstitial deletion of 13q22 in several patients with HD; and synteny between the murine locus for *piebald-lethal* (*sl*), a model of aganglionosis, and 13q22 in humans. Subsequently, an *EDNRB* missense mutation was identified in the Mennonite kindred (W276C) [24, 25]. Both *EDNRB* and *EDN3* were screened in large series of isolated HD patients, and *EDNRB* mutations were identified in approximately 5% of the patients. It is worth mentioning that the penetrance of *EDN3* and *EDNRB* heterozygous mutations is incomplete in those HD patients, de novo mutations have not hitherto been observed, and that S-HD is largely predominant [26].

SOX10

The last de novo mouse model for WS4 in human is *dominant megalon* (*Dom*). The *Dom* gene is *Sox10*, a member of the SRY (sex determining factor)-like, high-mobility group (HMG) DNA-binding proteins. Subsequently, heterozygous *SOX10* mutations have been identified in familial and isolated patients with WS4 (including de novo mutation) with high penetrance [27].

Clinical Signs/Symptoms

The clinical symptoms of HD usually start at birth with the delayed passage of meconium. More than 90% of term neonates, and <10% of children with HD, pass meconium in the first 24 h of life [1, 28, 29]. Thus, HCSR must be suspected in any full-term infant who does not pass meconium in the first 24 h of life and in the premature infants who have excessively delayed the passage of meconium (7–8 days) [29]. Failure of the distal bowel to relax and allow the passage of stool leads to functional obstruction and to secondary dilatation of the bowel proximal to the aganglionic segment. Affected children may present with severe dysmotility causing obstructive symptoms, ribbon-like stools, and frequently, failure to thrive. In >90% of affected patients, the symptoms start during the neonatal period, and in the majority, the diagnosis is made during the first 3 months of life, whereas <1% are diagnosed during adult life [30].

In infants and children, the presentation is often less dramatic and may not mimic acute intestinal obstruction. Severe constipation and recurrent fecal impaction are more common. Physical examination reveals a distended abdomen and a contracted anal sphincter and rectum in most children. The rectum is devoid of stool except in cases of short-segment aganglionosis. As the finger is withdrawn, there may be an explosive discharge of foul-smelling liquid stools, with decompression of the proximal normal bowel.

Complications

Over the past four decades, enterocolitis has been a major cause of morbidity and mortality in infants and children with HD. The mean incidence is 25%, but the range is great (from 17% to 50%) and may be differently estimated depending on the way it is diagnosed. Mortality rates range from 0% to 33%, probably reflecting differences in the diagnostic criteria [31]. Mortality also appears to be associated with other factors, such as trisomy 21. Pathogenesis is thought to be related to fecal stasis with proliferation of colonic bacteria, and therefore, a delayed HD diagnosis seems to be a significant risk factor [32]. The classic clinical manifestations described for enterocolitis include abdominal distension, explosive diarrhea, vomiting, fever, lethargy, rectal bleeding, and shock [33]. Abdominal radiographs show the Intestinal "cut-off" sign in the rectosigmoidal region with absence of air distally. Other common findings are small bowel dilatation in 74% and multiple air-fluid levels [34]. Because of the risk of perforation, contrast enema should not be performed in the presence of clinical enterocolitis.

Postoperative enterocolitis has been associated with fairly high rate of mortality in several series. In fact, when examining the deaths related to HD, several groups found that approximately 50% of deaths resulted from complications directly related to an enterocolitis episode [35, 36].

Rectal washouts should be the initial approach in the care of a child, regardless of age, who presents with enterocolitis. Along with washouts, intravenous antibiotics or oral metronidazole (in mild cases) should be used. Should the disease process fail to improve or the infant's condition deteriorate, the performance of a leveling colostomy should be considered [35, 36].

Diagnosis

For diagnosis of HD, the subjects' history is crucial. The main elements to obtain are: the age of the appearance of symptoms; whether the passage of meconium has been normal or delayed; and whether the child presented with episodes of functional intestinal obstruction. In addition, a functional (idiopathic) megacolon must be ruled out. A clinical comparison of functional and congenital megacolon is reported in Table 22.1. When the history (early onset of constipation, absence of fecal soiling) and/or the physical examination (empty rectal ampulla) suggest an organic cause, anorectal manometry (ARM) should be performed. ARM evaluates the response of the internal anal sphincter to inflation of a balloon in the rectal ampulla [37]. When the rectal balloon is inflated, there is normally a reflex relaxation of the sphincter. The rectoanal inhibitory reflex is absent in patients

Table 22.1 Differentiating types of megacolon in children

Signs and symptoms	Functional fecal retention (acquired)	Colonic neuromuscular disorders (congenital)
Soiling	Common	Rare
Obstructive symptoms	Rare	Common
Large-caliber stools	Common	Rare
Stool-withholding behavior	Common	Rare
Enterocolitis	Never	Possible
Associated upper-GI Symptoms	Never	Common
Symptoms from birth	Rare	Common
Localization of stools	Rectum	Rectal and extra-rectal

with HD; there is no relaxation, or there may even be para-

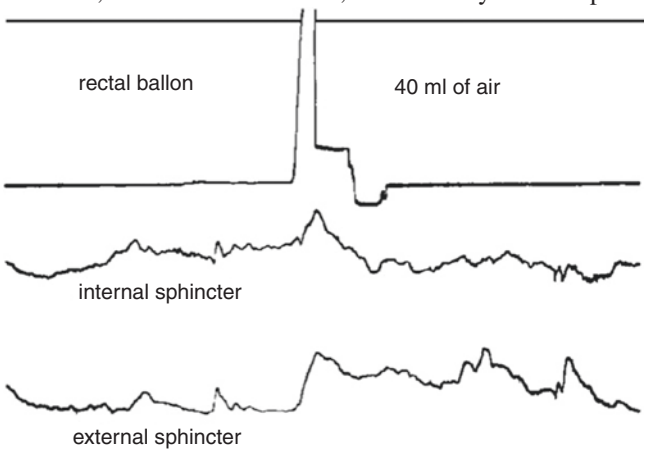

Fig. 22.1 Anorectal manometry in a 1-month-old boy with Hirschsprung's disease. Distention of a rectal balloon with air for 1 second produces no decrease of anal pressure

doxical contractions of the internal anal sphincter (Fig. 22.1). Although the absence of the rectoanal inhibitory reflex is specific for the diagnosis of HD, the role of ARM is still debated. A comprehensive systematic review by de Lorijn et al. [38] compared the diagnostic accuracy among rectal suction biopsy (RSB), ARM, and barium enema (BE) for the diagnosis of HD. Although RSB gave the highest mean sensitivity and specificity (93% and 98%, respectively), ARM showed similar values (91% and 94%). ARM has the advantages of being a less-invasive method without the exposure to ionizing radiation. The limitations include the need for the patient to be in a normal physiologic and quiet state to avoid possible artifacts [38, 39]. Because specificity is lower for ARM compared with RSB [37], ARM cannot reliably replace histology and biopsies. In 2013, a practical guideline was published by ESPGHAN GI committee and clearly stated that ARM should not be used as a sole diagnostic tool for HD in neonates and infants [40]; however; ARM is a useful screening test in older children presenting with chronic constipation and further symptoms suggesting HD (empty rectal ampulla, non-responsiveness to standard therapy, early-onset constipation). If the rectoanal inhibitory reflex is absent, these patients should be referred for RSB to confirm the diagnosis of HD. If the rectoanal inhibitory reflex is present, HD could be reasonably excluded [40]. BE is helpful in the assessment of a transition zone between aganglionic and ganglionic bowel and in giving an estimation of the length of an aganglionic segment. Demonstration of the transition zone is easier if no effort is made to cleanse the bowel (Fig. 22.2). In the newborn, dilatation of the proximal ganglionic bowel may not have developed, and radiological diagnosis may be more difficult. The sensitivity and specificity for recognition of a transition zone have been reported to be 80% and 76%, respectively [41]. The BE may not show a transition zone in cases of total colonic HD or may be indistinguishable from cases of functional constipation when ultra-short-segment HD is present. Therefore, a BE should not be performed as an initial diagnostic tool because it does not represent a valid alternative to RSB or ARM to exclude or diagnose HD; however, BE may have some use as an additional investigation in diagnosed cases to assess the length of the rectosigmoid aganglionic segment before surgery [40]. Nevertheless, the diagnosis requires histological evidence. The easiest means of obtaining adequate diagnostic tissue in rectal biopsies in infants is by rectal suction biopsy [42]. An accurate diagnosis is only possible if 2–3 suction biopsies are taken 2–3 cm above the dentate line and if they include enough submucosa [43]. Biopsies taken closer to the dentate line may be misleading because of a normal zone of submucosal hypoganglionosis or even aganglionosis [43]. The histological diagnosis is based on the demonstration of the total absence of ganglionic cells in the affected segment of the intestine with an overgrowth of large nerve trunks in the intermuscular and submucosal zone (Figs. 22.3 and 22.4). Acetylcholinesterase (AchE) activity in normal colon shows only few fibers in the lamina propria and muscolaris mucosae; in HD there is an increase in thick, knotted acetylcholinesterase-positive nerve fibers in the muscolaris mucosae and lamina propria and hypertrophied nerve trunks in the submucosa. Techniques for histopathological diagnosis include analysis of hematoxylin and eosin (H&E)-stained paraffin sections, snap-frozen sections stained for AchE, and immunostaining for neuronal markers. Knowles et al. stated that insufficient data exist to firmly recommend one approach over the other; however, the combination of AchE and H&E staining increases the diagnostic accuracy because it improves the identification of submucosal ganglion cells, as well as demonstrates the abnormal nerve fiber distribution in

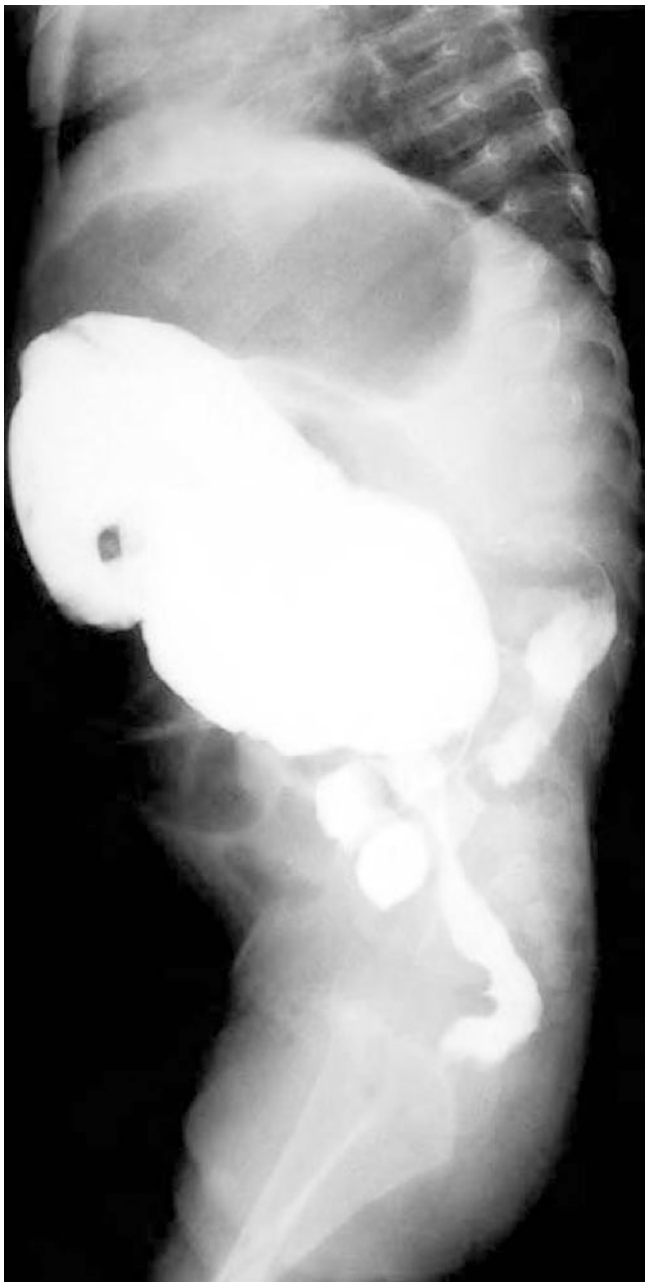

Fig. 22.2 Barium enema showing a long, narrowed segment in a child with Hirschsprung's disease

the left colon [43]. The potential value of immunohistochemistry has been evaluated in a number of studies. Calretinin is an immunohistochemical marker that may be a potential alternative to AchE [44, 45]. Calretinin is normally present in the perikarya and nerve processes of a subset of enteric ganglion cells. Immunoreactivity is lost in the aganglionic segment of HD [45]. A comparative study shows that calretinin is as sensitive and specific as AchE [46].

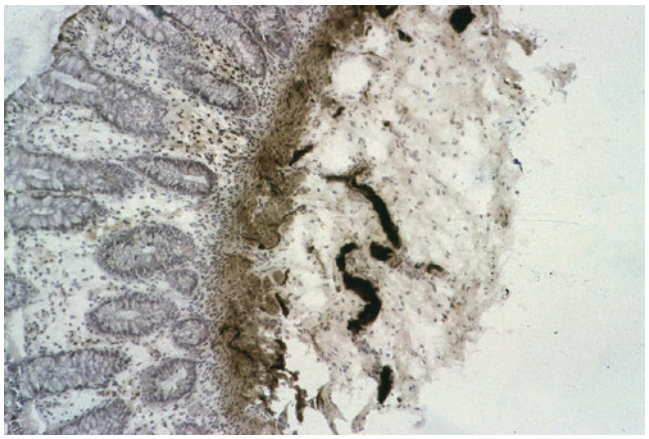

Fig. 22.3 Rectal suction biopsy in a child with functional constipation. Note the presence of clusters of neuron in the submucosa and acetylcholinesterase activity showing only few wispy fibers

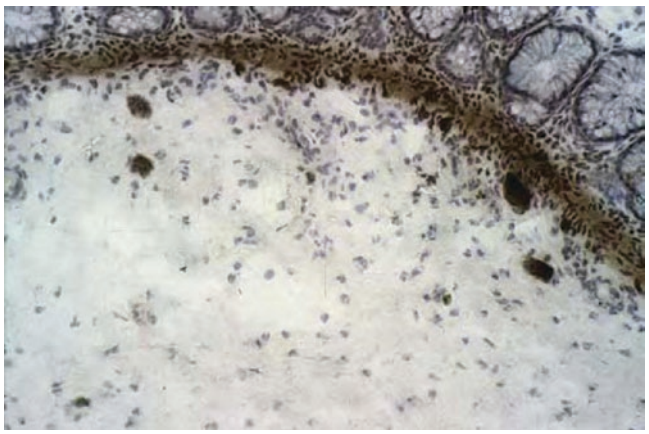

Fig. 22.4 Intense acetylcholinesterase activity in a patient with Hirschsprung's disease. Note the absence of neurons and the presence of increase in thick-knotted nerve fibers in the muscularis mucosae and lamina propria. In addition, hypertrophied nerve trunks are visible in the submucosa

Treatment

The treatment of HD consists of resecting the aganglionic segment of the rectum and colon, pulling down normally innervated bowel, and anastomosing this bowel at the anorectal region, while preserving the sphincter muscle. The previous gold standard of two- or three-stage pull-through with a preliminary stoma has slowly been progressively replaced by a one-stage approach in many centers [47–50]. More recently minimally invasive approaches to the one-stage pull-through have become popular. These consist of pull-throughs utilizing laparoscopic abdominal and pelvic mobilization of the rectum and the transanal Soave procedure, which does not include any intra-abdominal dissection [47–53]. The one-stage approach, either by laparotomy or by

combined laparoscopy and transanal dissection, has been advocated even in the newborn period.

The results of the one-stage approach in small infants appear to be at least as favorable as those in which a staged procedure with a colostomy was used. Recently, the use of the one-stage definitive procedure for small infants with HD has increased. One-stage pull-through procedures using laparoscopy appear to be associated with shorter hospital stays, shorter time until full feeding is reached, and superior cosmetic results [51–53].

Short- and Long-Term Prognosis

Despite adequate resection of the aganglionic segment, some patients may continue to have persistent bowel dysfunction [54]. Postoperative bowel dysfunction includes constipation, fecal incontinence, and a continuous risk of enterocolitis. An altered distribution and impaired function of ICC, resulting in defective generation of electrical pacemaker activity, have been suggested to be one of the mechanisms contributing to dysmotility in patients operated on for HD [55]. Investigations include barium studies to delineate strictures or leaks, and further biopsy to exclude residual aganglionic bowel. If the definitive operation fails because of an impassable stricture, disruption, or residual disease, further secondary surgery may be necessary, and a different operation may then lead to an acceptable result. Data are accumulating to indicate that HD, a disorder once known recognized exclusively to involve an aganglionic segment of distal colon, also affects motor function in other parts of the gut [54]. The variability in manifestations could reflect the heterogeneity of basic genetic defects now recognized as being responsible for the phenotypic expression of HD. Abnormalities in esophageal motility are common, and duodenal motor dysfunction is present in 48% of patients [5].

Miele et al. have reported a systematic study of various aspects of gastrointestinal motor function in children with HD long after removal of the aganglionic colonic segment, observing gastrointestinal symptoms, including vomiting, distension, and poor growth, persisted long after surgery [56]. Abnormalities in duodenal motor activity have also been observed in these children shortly after operation [57].

Intestinal Neuronal Dysplasia

Intestinal neuronal dysplasia (IND) or hyperganglionosis, a condition that clinically resembles HD, was first described by Meier-Ruge in 1971 [58]. It is often associated with HD and may cause failure of clinical improvement after resectional pull-through surgery. In 1983, Fadda et al. classified IND into two clinically and histologically distinguished subtypes, called type A and B. Type A occurs in less than 5% of cases and is characterized by congenital aplasia or hypoplasia of the sympathetic innervation, presenting acutely in neonatal period with episodes of intestinal obstruction, diarrhea, and bloody stools. Type B, which is clinically indistinguishable from HD: it is characterized by a malformation of the parasympathetic submucous plexus and accounts for more than 95% of cases of isolated IND [59].

The incidence of isolated IND varies from 0.3% to 40% of all suction rectal biopsies [60]. The incidence varies considerably among different countries; some investigators have a reported that 25–35% of patients with HD have associated IND [59, 61]. However, others rarely encountered IND in association with HD [62]. Part of this discrepancy may be due to the persisting confusion over the essential diagnostic criteria. For a long time, IND has been diagnosed on the basis of four histological criteria applied to acetylcholinesterase-stained suction rectal biopsies. In 1991, on the recommendations of a working party (the Consensus of German Pathologists), Bouchard et al. published diagnostic criteria for IND using suction rectal biopsy specimen. These comprised two obligatory criteria: hyperplasia of submucosal plexus and an increase in acetylcholinesterase-positive nerve fibers in the adventitia around submucosal blood vessels. Two additional criteria might be used: neuronal heterotopia and increased acetylcholinesterase-positive nerve fibers in the lamina propria [63]. The latest morphometric criteria are summarized as follows: >8 neurons/ganglion (so-called giant ganglia) in >20% of a minimum of 25 submucosal ganglia in patients older than 1 year [64]. However, concern has been expressed whether intestinal neuronal dysplasia can be safely diagnosed by mucosal and submucosal alteration alone, without myenteric plexus abnormalities. Submucosal hyperganglionosis may reflect a normal age-related phenomenon due to immaturity, with clinical and histochemical normalization after the first year of life. Furthermore, it has been reported that most of the patients with submucosal IND have a spontaneous clinical improvement which is sometimes associated with histological normalization [65, 66]. To date, submucosal intestinal neuronal dysplasia has been reported in several disorders such as intestinal malformations, meconium plug syndrome, cystic fibrosis, gastroschisis, pyloric stenosis, and inflammatory processes involving the gut. The high frequency of histological abnormalities in young infants may represent a normal variant of postnatal development rather than a pathological process. Investigations using more refined and morphometric methods in rectal specimens from infants and children without bowel disease are needed to define the normal range for different ages [66]. Therefore, most of the evidence suggests that the histological appearance of so-called IND is a normal variant related to age. Owing to the lack of sufficient normative data, IND remains a histological

description with poorly established clinical significance [40, 43]. Patients with IND have been subjected to multiple types of treatment; however, most patients with IND can be treated conservatively. If bowel symptoms persist after at least 6 month of conservative treatment, internal sphincter myectomy should be considered. The rapid AchE technique has been found to be of great value in determining the extent of IND intraoperatively [67].

Genetic Aspects

Studies have been performed to investigate the potential role of HD associated RET, GDNF, EDNRB, and EDN3 genes in the development of IND. They demonstrated that only three RET mutation were detected in patients with HD, no mutation in this gene was observed in IND and mixed HD/IND patients, HD and HD/IND patients showed overrepresentation of a specific RET polymorphism in exon 2, while IND patients exhibited a significantly lower frequency of the same polymorphism comparable with that of controls. These findings may suggest that IND is genetically different from HD.

A homozygous mutation of the EDNRB gene in spotting lethal (sl/sl) rats leads to HD phenotype with long segmented aganglionosis. The heterozygous (+/sl) EDNRB-deficient rats revealed more subtle abnormalities of the enteric nervous system (ENS): the submucous plexus was characterized by a significantly increased ganglionic size and density and the presence of hypertrophied nerve fiber strands, resembling the histopathological criteria for IND. Other animal model, likes Ncx/Hox11L.1-deficient mice, suggests that many other genes could be involved in the pathogenesis of IND [68].

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Intestinal Pseudo-Obstruction

23

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Introduction

The term "pseudo-obstruction" literally denotes the absence of a true mechanical occlusion. Intestinal pseudo-obstruction can be either acute or chronic in nature, reflecting the duration of obstructive symptoms [1, 2]. Chronic intestinal pseudo-obstruction (CIPO) was first described in 1958 by Dudley and colleagues to report a series of 13 patients with symptoms suggestive of intestinal occlusion. These patients underwent exploratory laparotomies, which failed to identify a mechanical cause for their symptomatology [3]. In subsequent years, the existence of this pathological entity in both adults and children was substantiated by a number of other clinicians [4–7].

In 2018, an ESPGHAN-led group of experts introduced the term "pediatric intestinal pseudo-obstruction" (PIPO) in order to distinguish pediatric from adult-onset CIPO. The aforementioned group of experts defined PIPO as a clinical entity "characterized by the chronic inability of the gastrointestinal tract to propel its contents mimicking mechanical obstruction, in the absence of any lesion occluding the gut." The group defined "chronic" as persistence of symptomatology for 2 months from birth or at least 6 months thereafter [8].

The pathophysiologic mechanism of PIPO is represented by abnormal antegrade propulsive activity of the gastrointestinal (GI) tract as a result of processes that affect its neurons, muscles, or interstitial cells of Cajal (ICC) [9]. This functional failure results in a number of clinical symptoms such as abdominal distention with or without abdominal pain, nausea, vomiting, and a reduced inability to tolerate enteral nutrition [10]. These symptoms are, however, nonspecific, and the condition can remain undiagnosed for a long period of time during which patients may undergo multiple diagnostic investigations and often repeated surgical explorations in an effort to identify the cause [10].

Although by definition the small intestine is always involved, any part of the GI tract can be affected in PIPO [1, 2, 8]. Esophageal involvement may lead to dysphagia from impaired peristalsis, in some cases akin to that seen in achalasia [11, 12]. Involvement of the stomach results in poor feed tolerance from gastroparesis suggested by the presence of delayed gastric emptying, while the large bowel by delayed colonic transit and constipation and the anorectum by sphincter dysfunction and defecation disorders [1].

This chapter focuses on various aspects of PIPO and attempts to address areas of controversy by exploring the most recent advances in the overall approach and management of this clinical entity.

Epidemiology

PIPO is a rare disease with scanty epidemiological data and poorly defined incidence and prevalence in both adult and pediatric populations. One of the few initiatives to elucidate its epidemiology suggested that approximately 100 infants are born in the USA every year with PIPO, suggesting an incidence of approximately 1 per 40,000 live births [13, 14].

Adult studies reveal that the disease is more frequent in females [15–17]. In a national survey conducted in Japan, 138 cases of chronic intestinal pseudo-obstruction were identified, with an estimated prevalence of 1.0 and 0.8 cases and incidence of 0.21 and 0.24 cases per 100,000 males and females, respectively [18]. Moreover, a recently published nationwide survey for PIPO in Japan revealed that the prevalence of PIPO, among children younger than 15 years, was 3.7 per one million children. In the aforementioned population, 56.5% of children had developed PIPO during the neonatal period [19].

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Undoubtedly, the development of national registries is of paramount importance to delineate more precise epidemiologic characteristics of this orphan clinical entity.

Classification

Classification of PIPO is challenging. Conditions can be classified by whether they primarily affect intestinal nerves (neuropathy), smooth muscle (myopathy), or ICC (mesenchymopathy) and can be further subdivided into primary or secondary, congenital or acquired, mode of inheritance or what part of the GI tract is involved. Where classification is not possible, they are defined as idiopathic. In truth, there is a considerable overlap [1, 2, 8].

In primary PIPO, the disease is usually localized to GI tract, whereas in secondary cases, there is a systemic disorder that affects GI tract motility. It must be noted though that in some cases of primary PIPO extra-GI involvement may also be present, such as the urinary tract (hollow visceral myopathy and megacystis microcolon intestinal hypoperistalsis syndrome), the nervous system (central, peripheral, autonomous), and/or mitochondria (mitochondrial neurogastrointestinal encephalomyopathy, MNGIE) [2, 20–22]. Table 23.1 depicts the classification of PIPO. In children the disease may manifest with symptoms either during the neonatal period (neonatal-onset form) or later (infantile or late-onset form); the majority of PIPO cases are congenital and primary, whereas in adults secondary forms of CIPO (mostly due to systemic disease) are more frequent [8, 23]. Based on histological findings, both primary and secondary PIPO can be further categorized into neuropathies, myopathies, and mesenchymopathies [24–29].

Table 23.1 Classification of Pediatric Intestinal Pseudo-obstruction [8]

Primary PIPO
Sporadic or familial forms of myopathy and/or neuropathy and/or mesenchymopathy that relate to disturbed development, degeneration, or inflammation [7, 20, 28–51]
Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) and other mitochondrial diseases [52–54]
Hirschsprung's disease (e.g., total intestinal aganglionosis)[a] [55–57]
Neuropathy associated with multiple endocrine neoplasia type IIB [58–60]
Secondary PIPO
Conditions affecting GI smooth muscle
Rheumatological conditions (dermatomyositis/polymyositis, scleroderma, systemic lupus erythematosus, Ehlers–Danlos syndrome) [61–72]
Other (Duchenne muscular dystrophy, myotonic dystrophy, amyloidosis, ceroidosis, or alternatively reported as brown bowel syndrome) [73–83]
Pathologies affecting the enteric nervous system (familial dysautonomia, primary dysfunction of the autonomic nervous system, neurofibromatosis, diabetic neuropathy, fetal alcohol syndrome, post-viral-related chronic intestinal pseudo-obstruction, e.g., CMV, EBV, VZV, JC virus) [84–99]
Endocrinological disorders (hypothyroidism, diabetes, hypoparathyroidism, pheochromocytoma) [100–104]
Malrotation or gastroschisis [105–107]
Neuropathy post neonatal necrotizing enterocolitis [108]
Idiopathic (i.e., where forms of PIPO classified as above do not, as yet, have a defined etiopathogenesis)

CMV cytomegalovirus, *EBV* Epstein–Barr virus, *VZV* varicella-zoster virus, *JC* John Cunningham, *GI* gastrointestinal
[a]Needs to be excluded in all cases of PIPO

Etiology and Pathophysiology

The integrity of GI sensorimotor function relies on precise coordination between the autonomic nervous system, enteric nervous system (ENS), ICC, and smooth muscle cells. Any noxious stimulus, irrespective of its origin and etiology, that affects the neuromuscular elements and control of GI tract can lead to impaired peristalsis and the stasis of luminal contents [1]. A variety of disorders and pathophysiological mechanisms can potentially affect the structure or function of the neuromuscular elements of the GI tract and lead to PIPO (Table 23.1) [8]. Neurological (e.g., multiple endocrine neoplasia (MEN) type IIb, familial dysautonomia) and metabolic (e.g., diabetes mellitus) conditions may affect the extrinsic GI nerve supply [23]. Neurotropic viruses may evoke an inflammatory process targeting both the ENS and extrinsic neural pathways [97]. Paraneoplastic syndromes may also exert a destructive effect on the ENS by initiating an inflammatory process that targets the neurons of ganglia located in the submucosal and myenteric plexuses. This is mediated by both a cellular infiltrate and production of circulating antineuronal antibodies [23, 109]. Some pathologies (e.g., muscular dystrophy) may target enteric smooth muscle fibers, whereas others such as dermatomyositis, scleroderma, Ehlers–Danlos syndrome, and radiation enteritis may distort both ENS and gut smooth muscle leading to a mixed neuromyopathic disorder [14, 110, 111]. Finally, although entities such as celiac disease, hypothyroidism, hypoparathyroidism, and pheochromocytoma presumably cause PIPO by affecting the GI neuromuscular integrity, the exact mechanism is not fully understood.

Genetics

Although there has been considerable progress, the elucidation of the genetic basis of PIPO has been rather limited. The majority of PIPO cases are sporadic [8]. Some familial cases of PIPO have been recognized, but there appear to be several patterns of inheritance, perhaps reflective of the great heterogeneity of PIPO conditions. Both autosomal dominant and

recessive modes of inheritance have been described for neuropathic and myopathic types of PIPO [5, 15, 16, 110, 112]. More specifically, rare autosomal dominant mutations in the *SOX10* gene, which encodes a transcription factor important in ENS development, result in a PIPO clinical phenotype along with features such as sensorineural deafness and pigmentary anomalies [113, 114]. Homozygosity on the region 8q23–q24 has been implicated in the pathogenesis of an autosomal recessive form of PIPO characterized by severe GI dysmotility, Barrett's esophagus, and cardiac anomalies [115, 116].

X-linked inheritance (locus Xq28) with recessive transmission has been described in PIPO [17, 117, 118]. Mutations of filamin A *(FLNA)* and L1 cell adhesion molecule *(L1CAM)* genes, which are both located on chromosome Xq28, result in predominantly myopathic and neuropathic forms of PIPO, respectively. Additional involvement of the central nervous system, heart (patent ductus arteriosus), and blood (thrombocytopenia) in both conditions has also been described [118–120].

Mutations in mitochondria are increasingly implicated in PIPO. Mutations in the thymidine phosphorylase gene (*TYMP*, also termed as endothelial cell growth factor-1, *ECGF1*), or in the polymerase-γ gene (*POLG*) result in recessive myopathic forms of PIPO. The former is the cause of MNGIE, whereas the latter leads to a form without encephalopathy. Apart from the GI dysmotility, MNGIE is characterized by severe malnutrition, opthalmoplegia, and leucoencepalopathy on brain MRI [121–123]. Furthermore, mutations in the following genes, *actin G2* [44], *RAD21* [124], and *SGOL1* [125], have been identified in recessive forms of PIPO with an associated syndromic phenotype.

Of note, with the advancement in genetic testing, novel mutations (MYLK, LMOD1, MYL9, MYH11, PDCL3, and ACTG2 variants) were identified and were subsequently related to the etiopathogenesis of megacystis microcolon intestinal hypoperistalsis syndrome [126–132].

Histopathology

In adults, GI histology is reported to be normal in approximately 10% of CIPO cases, while in the experience of the authors, this figure is likely to be higher in children. However, its role in PIPO remains crucial in order to inform prognosis and also guide further investigations for systemic diseases that require specific treatment; therefore, an adequate full-thickness biopsy is recommended whenever surgery is being considered [29]. Recent initiatives are addressing a more standardized and hopefully effective histological approach to diagnosis in GI motility disorders such as PIPO [29, 133, 134].

On the basis of histology, PIPO is classified into neuropathy, myopathy, or mesenchymopathy [29, 135]. However, mixed forms (e.g., neuromyopathy) are also recognized [29].

Neuropathies and myopathies can be further subdivided into inflammatory and degenerative. Inflammatory neuropathies are characterized by an infiltration of T lymphocytes and plasma cells in the myenteric plexuses (myenteric ganglionitis) and neuronal axons (axonopathy) [136–138]. It has been proposed that five or more lymphocytes per ganglion are required for the diagnosis of myenteric ganglionitis [137, 139]. Of note, patients with lymphocytic infiltration of the myenteric plexuses may also develop increased titers of antinuclear antibodies (ANNA-1/anti-Hu, anti-voltage-gated potassium channel or VGKC) [49, 140–142]. These immunologic responses may result in neuronal degeneration and loss by activating apoptotic and autophagic mechanisms [143]. Infiltration of the myenteric ganglia with other cells such as eosinophils and mast cells has been described, but their exact clinicopathological significance is yet to be clarified given limited data [144–146]. All these data support the role of the immune system in the pathogenesis of inflammatory PIPO [135, 147].

Degenerative neuropathies are poorly understood given the limited amount of available data [133, 147–149]. Main histopathologic characteristics of this group include a decrease in the number of intramural neurons along with changes in nerve cell bodies and axons [46, 150]. It has been postulated that apoptotic mechanisms are involved in the degenerative process potentially caused by aberrant calcium signaling, mitochondrial disorders, production of free radicals, and abnormalities in the function of glial cells [151, 152].

Similarly to neuropathies, myopathies are also divided into inflammatory and degenerative. Inflammatory myopathies, also reported by the term "leiomyositis," are characterized by infiltration of T lymphocytes into both the circular and longitudinal enteric muscle layers. This process if not treated appropriately with immunosuppressive agents may lead to a severe clinical picture of PIPO [121, 123].

The histopathologic findings in degenerative myopathies include smooth muscle fiber vacuolization and fibrosis [153]. Diverticula may also be present especially if the longitudinal muscle coat is more affected compared to the circular muscle layer [147, 154].

Novel immunohistochemical techniques, such as smooth muscle markers, namely, smoothelin, smooth muscle myosin heavy chain, and histone deacetylase 8, may reveal histiopathologic subtleties otherwise not detectable with conventional immunostaining and histochemistry methods [29].

Mesenchymopathies are defined by ICC abnormalities (decreased density of ICC network, intracellular abnormalities) and have been demonstrated in patients with chronic intestinal pseudo-obstruction [8, 9, 155]. Although sufficient

data exist regarding their role in the pathogenesis of diabetic gastroparesis, further research is required regarding ICC involvement in the etiopathogenesis of other GI motility disorders [26].

Clinical Picture

In a few cases, the diagnosis of PIPO is suggested in utero by ultrasonographic findings of polyhydramnios, abdominal distention, and megacystis; however, the majority of cases present in the neonatal period or early infancy [9, 10, 156]. The symptomatology varies according to the age at diagnosis and the part of the GI tract, which is primarily affected. Approximately, one-third of children with congenital PIPO (myopathic and neuropathic) have intestinal malrotation [156]. Cardinal signs and symptoms of PIPO include those of obstruction, namely abdominal distention (88%), vomiting (72%, which can be bilious), and constipation (61%). Abdominal pain (44%), failure to thrive (31%), and diarrhea (28%) may also be part of the clinical picture [20, 122].

Dehydration (which can be severe) and malnutrition are often underdiagnosed especially given that weight can be an unreliable measure due to pooling of significant volumes of fluid (third spacing) within distended gut loops. Intraluminal gut content stasis can also lead to small bowel bacterial overgrowth which can further exacerbate symptoms of diarrhea and abdominal distention [62].

PIPO may also manifest with extraintestinal signs and symptoms, such as recurrent urinary tract infections or neurologic abnormalities [155]. Furthermore, patients may complain of symptoms indicative of an underlying disorder that accounts for secondary PIPO (e.g., proximal muscle weakness in dermatomyositis) [10, 156].

The clinical course of PIPO is characterized by exacerbations and remissions, where the former can be precipitated by a number of factors such as surgery, general anesthesia, infections, and emotional stress [8]. In the most severe cases, the natural course of the disease leads to worsening intestinal function and ultimately to intestinal failure [8]. This is especially true in cases where the diagnosis and/or institution of appropriate treatment has been delayed.

Diagnosis

PIPO should be suspected in children with early onset, chronic, recurrent, or continuous signs of intestinal obstruction especially where a surgical cause cannot be established (e.g., repeated "normal" exploratory laparotomies). The diagnosis of PIPO should follow a structured algorithm as proposed by the ESPGHAN-led expert group [2, 156, 157]. Although a detailed history, clinical examination, and laboratory tests may suggest the presence of PIPO, or help elucidate its cause, the ESPGHAN-led expert group proposed that the definitive diagnosis requires at least two out of the four following criteria [158]:

(i) Objective measure of small intestinal neuromuscular involvement (manometry, histopathology, transit studies)
(ii) Recurrent and/or persistently dilated loops of small intestine with air fluid levels
(iii) Genetic and/or metabolic abnormalities definitively associated with PIPO
(iv) Inability to maintain adequate nutrition and/or growth on oral feeding (needing specialized enteral nutrition and/or parenteral nutrition support)

Careful clinical history and physical examination may help in defining the onset, the severity and progression of the disease, and the part of the GI tract primarily affected, and they also provide useful information regarding associations (e.g., family history), potential secondary causes (e.g., medications), and complications (e.g., dehydration). Laboratory tests [e.g., serum electrolytes, thyroid-stimulating hormone (TSH), lactic acid, specific autoantibodies] are useful in cases of secondary PIPO and in order to assess the clinical state of the patients admitted acutely or undergoing a diagnostic protocol.

Imaging

Plain abdominal radiographs may demonstrate a dilated GI tract, with air-fluid levels, whereas contrast GI series can reveal anatomical abnormalities (e.g., malrotation, microcolon) and exclude the presence of gut occlusive lesions (Fig. 23.1a) [159]. It needs to be kept in mind that a water-soluble substance should be used instead of barium in order to prevent flocculation and inspissation of the contrast material.

Novel imaging modalities such as cine MRI have been recently performed with promising results in adult series, but there are no data regarding their applicability and usefulness in pediatrics [160, 161].

Endoscopy

Endoscopy may identify fore- or hindgut mechanical occlusion previously missed on radiology, and it allows duodenal biopsies to exclude mucosal inflammation [9, 162, 163]. Novel techniques (e.g., natural orifice transluminal endoscopic surgery—NOTES) may revolutionize the role of endoscopy in the diagnosis of gut motility disorders by

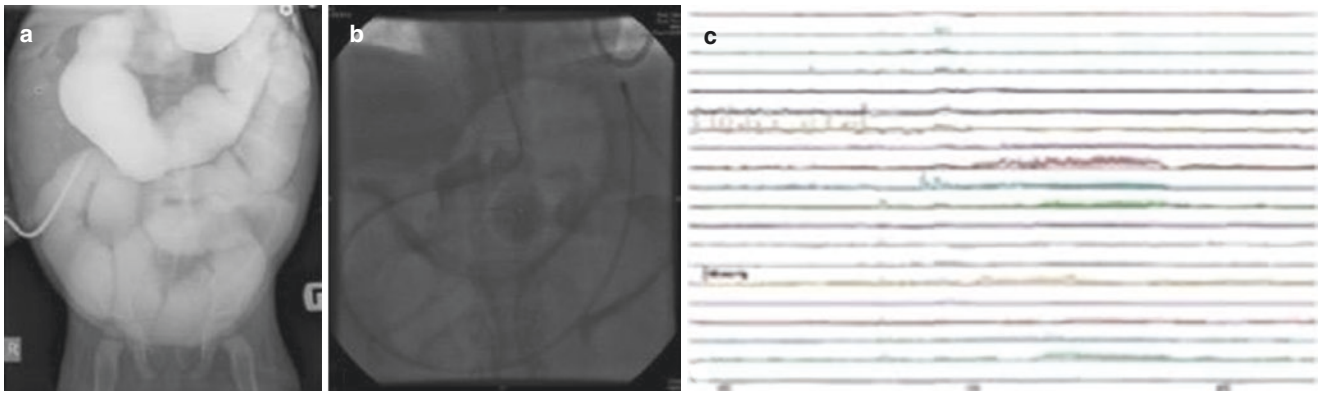

Fig. 23.1 Investigation findings of a 3-year-old boy with a history of recurrent episodes of abdominal distension and vomiting since the neonatal period, and now showing a marked reduction in enteral feed tolerance. (**a**) Contrast follow-through study (administered via gastrostomy) showing filling of grossly dilated small intestinal loops, without any apparent hold up or change in caliber. (**b**) Plain abdominal radiograph taken following placement of antroduodenal manometry catheter into the same patient performed under fluoroscopic guidance. The tip of the catheter has been advanced beyond the duodenojejunal junction to facilitate optimal manometric recording of both the stomach and small intestine. (**c**) Antroduodenal manometry tracing from patient showing the presence of some gastric antral contractions and a migrating motor complex (phase III activity) passing down the small intestine. The amplitude of small intestinal contractile activity is very low (not exceeding 20 mmHg) suggesting a diagnosis of myopathic chronic intestinal pseudo-obstruction

providing the ability of full-thickness biopsy sampling in a safe and minimally invasive way [164].

Motility Investigations

These studies are performed to assess the GI motility and to define the underlying pathophysiologic process, and these studies form the hallmark of diagnosis in pediatrics. Investigations include GI manometries (esophageal, antroduodenal, colonic, anorectal), GI scintigraphy (e.g., gastric emptying, colonic transit), electrogastrography, and radiopaque markers. The usefulness of novel technologies, such as SmartPill, remains to be determined [165–167].

Although in children with PIPO the involvement of GI may be generalized, the small intestine is always affected; thus, antroduodenal manometry remains the most discerning test, and its optimal placement is pivotal (Fig. 23.1b) [168–170]. Neuropathic cases manifest with uncoordinated contractions, which are of normal amplitude, whereas in myopathic PIPO, motor patterns have normal coordination; however, the amplitude of intestinal contractions is low (Fig. 23.1c) [171, 172]. Additionally, manometry may facilitate the dynamic assessment of potential pharmacotherapeutic options and feeding strategies (e.g., feasibility of oral or enteral feeds) as well as indicate disease prognosis [156, 173, 174].

In the most challenging cases, exploratory surgery (laparotomy or laparoscopic-assisted procedures) may be required to definitively exclude mechanical obstruction from PIPO. One however should bear in mind that surgery may precipitate a pseudo-obstructive episode and may also lead to adhesions formation, which can further complicate future diagnostic or therapeutic procedures. Where possible, investigations and then diagnostic/therapeutic surgery should be performed in timeline sequence and in referral center.

Histopathology along with genetics can also be very useful in establishing or confirming the diagnosis of PIPO, highlighting the underlying pathophysiologic process, thus aiding the overall management.

Differential Diagnosis

PIPO has to be differentiated from mechanical obstruction; the latter is usually characterized by marked abdominal pain (in keeping with the abdominal distention), specific radiologic signs, and manometric patterns [111, 175]. Acute functional obstruction (e.g., postoperative ileus), functional GI disorders (e.g., rumination syndrome), and pediatric condition falsification should be considered and appropriately investigated and managed [9].

Treatment

The therapeutic approach in PIPO is threefold as it aims to (i) preserve growth and development by maintaining adequate caloric intake, (ii) promote GI motility with combined medical and surgical interventions, and (iii) treat disease-related complications or underlying pathologies that cause secondary PIPO. Despite the limited effects of the currently applied therapeutic modalities, refinements and evolution in nutritional, medical, and surgical strategies have considerably

improved the overall management of PIPO [155]. Acute management of episodes of pseudo-obstruction is generally treated conservatively by nil by mouth, intravenous fluid, and drainage of stasis through nasogastric (NG) tube or preformed ostomies. Careful attention to fluid and electrolytes is imperative.

Nutrition

The role of nutrition in PIPO is of paramount significance as it is well known that gut motility improves with optimal nutritional support and declines in the face of under- or malnutrition [176–180]. In the long term, approximately one-third of PIPO patients require either partial or total parenteral nutrition, another third require a degree of intragastric or intra-enteral feeding, whereas the remaining children are able to tolerate sufficient oral nutrition. However, within all of groups, patients able to tolerate feeds may require some dietary modification in order to maintain enteral nutrition and avoid bezoar formation (e.g., bite and dissolvable feeds, restriction diets, hydrolyzed formula).

Although parenteral nutrition is lifesaving, it is associated with significant risk of complications, such as central line infections and liver disease, and therefore maintaining patients on maximally tolerated enteral nutrition is always strongly encouraged [169, 178]. In the more severe PIPO cases, continuous rather than bolus feeds administered via a gastrostomy or jejunostomy may be better tolerated particularly in children with impaired gastric motor function [8, 181–184].

Medications

The therapeutic role of drugs in PIPO patients is mainly limited to the control of intestinal inflammation, suppression of bacterial overgrowth, and promotion of GI motility [185].

Prokinetics (e.g., metoclopramide, domperidone, erythromycin, azithromycin, octreotide, neostigmine, pyridostigmine) usually combined with antiemetics (e.g., promethazine, ondansetron) have been used in an attempt to improve the GI motor function and reduce the severity of nausea and vomiting [186–191]. The use of some of these agents is limited by variable efficacy and unacceptable extraintestinal side effects (e.g., metoclopramide, neostigmine). The best-studied and tested prokinetics, that is, cisapride and tegaserod have been withdrawn from the market due to safety concerns [169, 192]. The need for new prokinetics with increased safety and efficacy has resulted in new products (e.g., prucalopride, aprepitant, ghrelin), but there are limited data of their use in pediatric PIPO, further impacted on by restricted availability and licensing [178, 193, 194].

Undoubtedly, current medical regimens for PIPO are based on limited literature and/or expert opinion (e.g., combined use of octreotide and erythromycin) and are yet to be tested in future in the context of controlled trials [178, 193].

Surgery

Surgery remains a valuable intervention on patients with PIPO as it has a multidimensional role in both the diagnostic (e.g., full thickness biopsies) and therapeutic processes (e.g., insertion of feeding tubes, formation of decompressing ostomies such as gastrostomy, ileostomy) [195, 196].

Indeed, adequate bowel decompression is crucial not only in providing symptomatic relief by reducing the frequency and the severity of pseudo-obstructive episodes but also in limiting further deterioration of the intestinal motor activity secondary to chronic distention, and in enhancing the tolerance of enteral feeding [197]. Long decompression enteral tubes and extensive bowel resections are approaches mainly reported in adult CIPO cohorts but remain untested in terms of practicality, efficacy, and safety in pediatrics [198–201]. Rate of significant surgical complications, such as stoma prolapse, infection, and leakage can be significant.

Novel surgical methods involve implantation of devices providing electrical pacing of the GI neuromusculature, but data in children are scanty and limited [8, 15–17, 144].

Small bowel transplantation remains the only definitive cure. Recent advances in both surgical techniques (e.g., multivisceral transplantation) and immunosuppression strategies have resulted in improved outcomes and survival as reported by centers with the relevant expertise showing a survival rate of 50% at 3 years [13, 25, 31, 202–206].

Natural History and Prognosis

Both pediatric and adult chronic intestinal pseudo-obstructions have a severe clinical course, characterized by repetitive relapses and remissions. Unfortunately, the low index of suspicion among physicians along with lack of well-defined diagnostic criteria and readily available facilities in performing specialized diagnostic tests (e.g., manometry) often accounts for delays in the diagnosis and repetitive unnecessary investigations and surgery [206].

The majority of the patients complain of symptoms, which progressively worsen and impact upon the tolerance of enteral nutrition and increasing reliance on total parenteral nutrition [179, 180]. The latter in conjunction with disease-related adverse events (e.g., central line infections, impairment of the liver function, immunosuppression after small bowel transplantation, surgical procedures) accounts for

high morbidity, poor quality of life, and mortality rates up to 30% [13, 25, 31, 202–206].

Despite recent diagnostic and therapeutic advances, PIPO in children remains a serious, life-threatening disease with significant impact on the well-being not only of patients themselves but also of their families [206].

Summary

PIPO is a debilitating disease with poorly defined etiopathogenesis, which is reflected on the limitations encountered in both the diagnostic process and therapeutic management. Clearly, multinational initiatives are required to raise awareness and evolve current diagnostic modalities and therapeutic options.

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Gastrointestinal and Nutritional Problems in Neurologically Impaired Children

24

Paolo Quitadamo and Annamaria Staiano

Introduction

The increasing survival of children with severe central nervous system damage accounts for a major challenge for medical care. Although the primary problems for individuals with developmental disabilities are physical and mental incapacities, several clinical reports have indicated that brain damage often results in significant gastrointestinal dysfunction [1–4]. The enteric nervous system contains more neurons than the spinal cord, and thus, it is not surprising that insults to the central nervous system may affect the complex integrated capacities underlying feeding and nutrition [5].

The increased awareness of such conditions, together with a better understanding of their etiology and interplay, is essential to achieve an optimal global management of this group of children.

Feeding and Nutritional Aspects

Historically, severe malnutrition has been accepted as an unavoidable and irremediable consequence of neurological impairment. Poor nutritional status was often marked by linear growth failure, decreased lean body mass, and diminished fat stores [6, 7]. Over the past two to three decades, multidisciplinary feeding programs providing comprehensive evaluation and treatment of feeding disorders in children with developmental disabilities have been instrumental in improving the nutritional status and quality of life and reducing the hospitalization rates [8]. Nutritional assessment and nutritional interventions in neurologically impaired children are a challenge for physicians but should be part of the child's comprehensive care and rehabilitation.

Assessment of nutritional status is the first step in the clinical nutritional evaluation of children with neurological impairment. In these children, measurement can be difficult and the references commonly used in pediatric patients tend to misinterpret undernutrition. Whenever possible, weight measurement should be obtained on a digital scale or, if the child is unable to stand, on a wheelchair scale [9]. Standing height or supine length can be used in children who can stand or lay down straight. However, accurate evaluation of stature may not be possible because of spasticity, joint contractures, or scoliosis. In children who are unable to stand upright due to skeletal deformity, alternative measurements for the height assessment should be segmental lengths, such as knee-heel length, tibia length, and ulnar length, assessed by sliding calipers [9, 10]. Special equations or charts can then be used to calculate the standing height [11]. Assessment of nutritional status in neurologically impaired children should not be based on weight and height measurements alone, but should include the evaluation of body composition [9, 10]. The three most commonly used measurements to calculate growth charts in typically developing children, such as weight-to-height ratio, height for-age, and weight-for-age, are poor predictors of body composition in this group of patients [9]. Weight measurements do not distinguish between muscle and fat mass percentages. Neurologically impaired children have higher fat percentages and lower lean masses than typically developing children. Body mass index is not recommended especially when derivative measures of body length are used [9]. Therefore, parameters to assess malnutrition and overnutrition in the handicapped child have to be adjusted. Children should be studied as for anthropometric parameters, body composition, bone status, and laboratory nutritional values [9, 12]. An algorithm for the suggested approach to the nutritional assessment of the neurologically impaired child (from ref. 9) is reported in Fig. 24.1.

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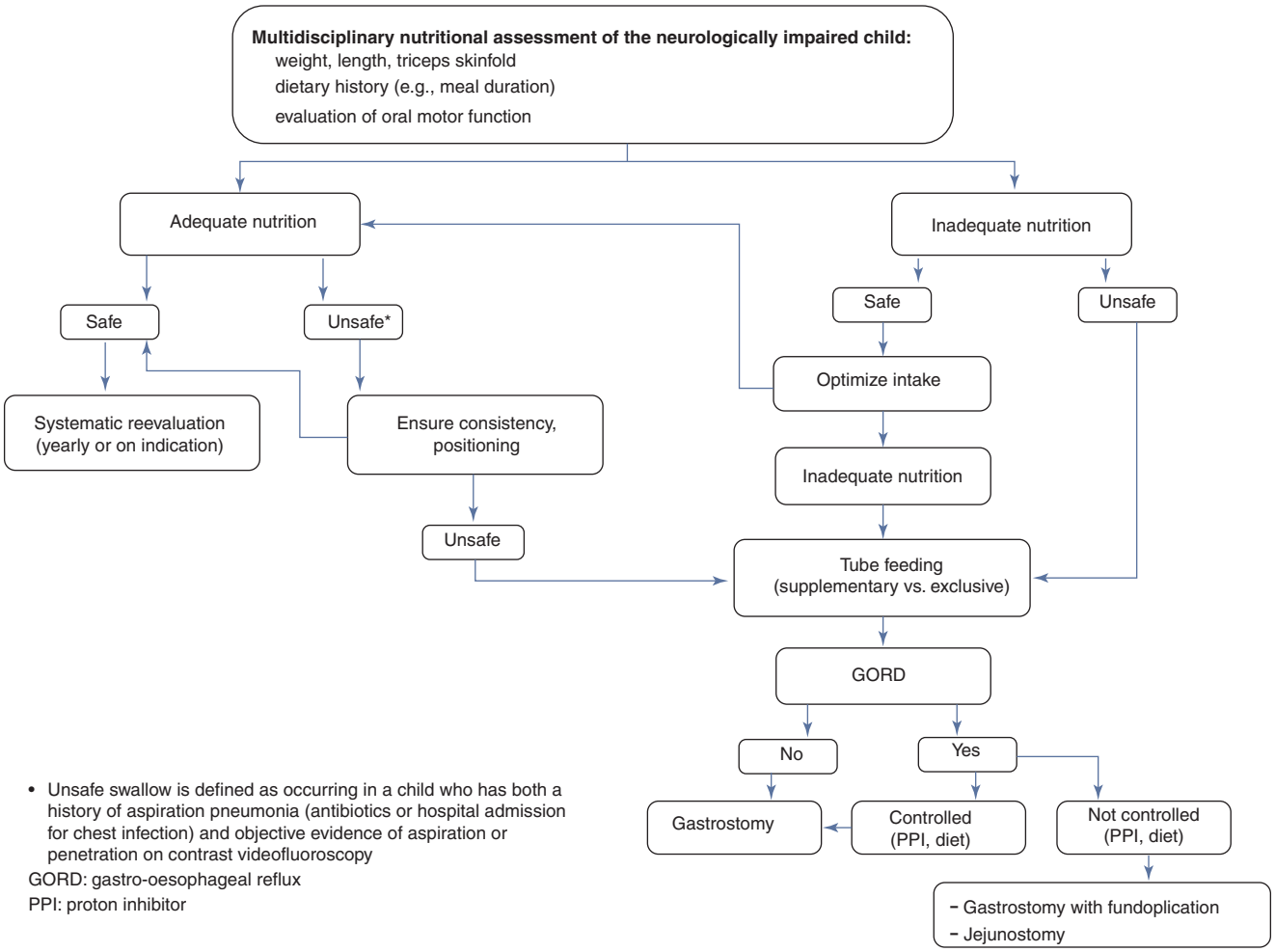

Fig. 24.1 Algorithm for the nutritional evaluation of the neurologically impaired child. (Reproduced from ref. 9 with permission)

The true prevalence of undernutrition in neurologically impaired children is unknown. It has been estimated that approximately one-third of them are undernourished and many exhibit the consequences of malnutrition [13]. Yet, the incidence and severity of malnutrition increases with the duration and the severity of neurological impairment [14–16]. Moreover, it should be considered that no single, universally accepted definition of undernutrition in children exists, neither in typically developing nor in neurologically impaired children [17, 18]. For clinical practice, ESPGHAN recommends the use of 1 or more red flag warning signs, including physical signs of undernutrition, such as pressure sores and poor peripheral circulation, weight for age z score <−2, triceps skinfold thickness <tenth centile for age and sex, mid-upper arm circumference or muscle area <tenth percentile, faltering weight, and/or failure to thrive [9]. These recommendations are mainly based on experts' opinion and low−/moderate-quality studies. Recently, they have validated some screening tools aimed to identify risk of feeding and swallowing difficulties or undernutrition earlier and to assess if further assessment is needed [19].

The predominant nutritional deficit in neurologically impaired children is in energy intake, with only 20% of these children regularly ingesting 100% of their estimated average requirement. Moreover, half of the children with severe disabilities consumed less than 81% of the reference nutrient intake for copper, iron, magnesium, and zinc, with that influenced by their large consumption of milk [20, 21]. Nutritional support is essential for the care of neurologically impaired children. Undernourished handicapped children might not respond properly to intercurrent diseases and suffer unnecessarily. On the other hand, restoring a normal nutritional status results in a better quality of life in many. Assessment of nutritional status requires a proper follow-up of height, body weight, and assessment of the standard deviation score. By so doing, negative changes are easily discovered and appropriate nutritional intervention can be initiated. An individualized intervention plan that accounts for the child's nutritional

status, feeding ability, and medical condition should be determined. Energy requirements must be individualized considering mobility, muscle tone, activity level, altered metabolism, and growth. The easiest and least invasive method to increase energy intake is to improve oral intake. Food caloric density may be increased by adding modular nutrients, modifying recipes or using high-calorie formulas. Children who cannot chew effectively may be able to receive the same foods blenderized into a puree of acceptable consistency. Those who can tolerate solids but not liquids can have commercial thickeners added to their fluids. Oral feeding skills may be improved with therapy, even if the results may be disappointing [22–24]. Adequate positioning of the child during meals and appropriate food temperature are furthermore important. However, oral intake can be maintained as long as there is no risk of aspiration, the child is growing well, and the time required to feed the child remains within acceptable limits.

When oral intake is unsafe, insufficient, or too time-consuming, enteral nutrition should be initiated. The type of enteral access will depend on the anticipated duration of enteral nutrition support as well as the clinical status of the child. Nasogastric tubes are minimally invasive but are easily dislodged and have local complications such as sinusitis, congestion, otitis, and skin irritation. Generally, nasogastric feeds should only be used for short-term nutritional support (less than 3 months). For long-term enteral nutrition support, a gastrostomy should be considered (Fig. 24.1). Gastrostomies are more invasive but are also more convenient and esthetically acceptable. Gastrostomy placement has been shown to reduce feeding time, food-related choking episodes, frequency of chest infections, and family stress and to improve weight and nutritional status significantly in children with severe neurologic impairment [25, 26]. However, percutaneous endoscopic gastrostomy (PEG) is not without complications or concerns. Minor catheter infections, perforation, and an overall lessened length of survival have been described in both adult and pediatric population [27–32].

The anatomy and function of the stomach should be carefully evaluated before the placement of the feeding tube. The coexistence of gastro-esophageal reflux (GER) may require a simultaneous fundoplication, and delayed gastric emptying must necessitate pyloroplasty or duodenal placement of the distal portion of the tube. Physiologically designed formulas of increased caloric and protein density can be used for gastric and nasogastric infusion, as palatability is no longer an issue. The choice between bolus and drip may depend on esophagogastric function, the volume to be delivered, or the home care needs of the child and his or her caregivers. Often patients may benefit from a combination of daytime bolus and nocturnal continuous feeds, the latter providing 30–50% of the child's nutrient needs thus allowing more freedom for daily activities. When safety of oral feeding is not an issue, these enteral techniques can merely supplement the child's own nutrition, with caregivers continuing to feed the child actively. This dual feeding method often provides great satisfaction to parents and caregivers, because the mealtime interaction is improved when there is no longer need for force-feeding of medication or nourishment.

Gastrointestinal Problems

Chronic gastrointestinal (GI) disorders are very common in children with neurological impairment, being reported a prevalence of up to 92% [33]. Dysphagia, rumination, GER, delayed gastric emptying, abdominal pain, and constipation have all been described in this group of children, potentially contributing to feeding difficulties and carrying challenging long-term management issues.

Dysphagia

Oral motor dysfunction is a frequent concomitant and often one of the first signs of neuromuscular impairment. Related swallowing problems have been shown to affect up to 90% of neurologically impaired children, being major contributors to malnutrition [1, 34]. This is not surprising since the development of oral-motor skills mirrors general neurological maturation and requires the coordination of the movement of several striated muscles in the mouth, pharynx and esophagus by six cranial nerves, the brain stem, and the cerebral cortex. In addition, anatomic abnormalities such as cleft palate, laryngeal clefts, and tracheoesophageal fistula may accompany neurologic deficits as part of a congenital or genetic syndrome. Dysphagia may manifest as distress during meals (including coughing, choking, and refusal of feeding), chronic or episodic aspiration-related respiratory disorder, and failure to thrive. Barium swallow, cine-swallow, radionuclide esophageal clearance scan, and esophageal manometry may all be of some help in the clinical assessment. Successful management of dysphagia is central to the child's well-being and ability to achieve his or her potential. Neurologically impaired children often show greater problems with liquid foods, thus requiring the use of thickener products. Oral motor exercise approaches using sensory modalities may help improving muscle strength and oral coordination. Nevertheless, in most cases, the presence of unsafe swallows and/or long-lasting distressed meals finally lead to the choice of enteral nutrition.

Gastro-esophageal Reflux

Several reports have demonstrated the high incidence of GER in neurologically impaired children. GER symptoms such as vomiting, rumination, and regurgitation are found in 20–30% of the intellectually disabled population [35]. GER-related iron deficiency anemia and hematemesis are noted in 10–20% of patients. In the Netherlands and Belgium in a large cohort of more than 1500 patients, a randomly selected intellectually disabled population was tested with pH-metry during 24 hours. A pathological pH test (defined as a duration of a pH of <4 for more than 4% of the measured time) was seen in 48% of cases [36]. These patients were subjected to endoscopy and reflux esophagitis was found in 96%: 14% had grade I esophagitis, 33% had grade II, 39% had grade III, and 13% had grade IV (Savary Miller classification). Barrett's esophagus was found in 14% and peptic strictures in 4% of cases. This study was repeated for the group under the age of 14, and similar findings were seen. In fact, GER disease was found even in the absence of overt symptomatology. Prolonged supine position, increased intra-abdominal pressure secondary to spasticity and scoliosis, a coexisting hiatal hernia have been supposed to be contributing factors to the increased frequency of GER. Central nervous system dysfunction, however, is likely to be the prime cause, being GER probably a part of the generalized dysmotility of the foregut, if not of the entire intestine. Decreased resting pressure and increased frequency of transient relaxations of the lower esophageal sphincter, together with motility abnormalities, are probably a consequence of neuromuscular incoordination.

Currently the most accurate way of diagnosing GER is 24-hour esophageal pH impedance recording, which allows not only to quantify the amount of reflux episodes but also helps in establishing the temporal relationship between GER and the symptom complex in question. The diagnostic work-up should then include upper GI endoscopy with multiple esophageal biopsies and upper GI barium study, in order to evaluate the mucosa and to look for the possible presence of strictures, diverticuli, or hiatal hernia. Radionuclide studies such as gastro-esophageal scintigraphy should also be performed, due to the higher incidence of delayed gastric emptying which may contribute to GER [37, 38]. An esophageal manometry evaluating visceral motility may be helpful to detect the underlying pathophysiological mechanisms, especially when surgery becomes necessary.

Although children with neurologic impairment are more likely to have intractable reflux and eventually require some surgical procedures, medical therapy should be tried first. When surgery becomes advisable, the Nissen fundoplication is currently the most widely used technique to strengthen the antireflux barrier and relief symptoms [39].

Constipation

Infrequent stool passage and hard bowel movements are very common in neurologically impaired children. The incidence of constipation was around 61% in a large cohort of mentally handicapped children in Dutch and Belgian institutions [40]. Total and sequential colonic transit times have been shown to be prolonged and delayed at the level of the left colon and rectum in this group of children, implying a probable defect in gut innervations [3]. The problem is usually exacerbated by prolonged immobility, inadequate fiber intake, and medications. Unfortunately, recognition and therefore effective management of constipation are often delayed because their other disabilities overshadow those related to defecation. Therapeutic approach needs to be tailored to the individual patient. Oral or rectal disimpaction should be followed by promotion of regular bowel habits, through dietary modifications, positioning, and use of medications. A significant number of children with neurological impairment need to be on chronic doses of laxatives, which are usually effective in enabling regular defecations. However, when medical treatment fails, a surgically placed appendicostomy should be considered.

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Cyclic Vomiting Syndrome

25

Katja Kovacic and BU K Li

Introduction

Despite improved characterization, recognition, and understanding of cyclic vomiting syndrome (CVS) in the past three decades, without a delineated pathophysiologic cascade, it remains classified as a functional gastrointestinal disorder. The hallmark symptoms described by Samuel Gee in 1882 remain applicable today and include stereotypical, severe episodes of vomiting punctuating symptom-free periods or baseline health [1]. Earlier clinical diagnosis has been facilitated by more specific consensus diagnostic criteria defined by the North American Society for Pediatric Gastroenterology, Hepatology & Nutrition (NASPGHAN; 2008) and Rome IV criteria (2016), the former being quantitatively more rigorous by requiring three to five vs two total episodes (Table 25.1) [2, 3]. Although there is considerable clinical overlap between migraine conditions such as abdominal migraines and CVS, the predominant and most consistent symptom during episodes best defines the illness, that is, abdominal pain is termed abdominal migraine, and conversely vomiting is denoted CVS. The continuum between CVS and migraine was suggested by Whitney already in 1898 and corroborated by other authors in 1998 [4, 5]. In a cross-sectional school survey in Scotland, Abu-Arafeh described a probable developmental progression from CVS to abdominal migraine and migraine headaches (median ages 5, 9, and 11 years and comparative prevalence 1.9%, 4%, and 11%, respectively) [6]. This suggestion of a natural history that begins with CVS and ends with migraines reproduces initial reports by Barlow in 1984 who labeled this progression as the "periodic syndrome." [7] Although some experience all three phases, the majority trade CVS for migraines beginning at age 10 and an estimated 75% will develop migraine headaches by age 18 years.

Table 25.1 NASPGHAN and Rome IV diagnostic criteria

NASPGHAN
1. At least five attacks in any interval or a minimum of three attacks during a 6-month period.
2. Episodic attacks of intense nausea and vomiting lasting 1 h to 10 days, occurring at least 1 week apart.
3. Stereotypical pattern and symptoms in the individual patient.
4. Vomiting during attacks at least 4 times/h for at least 1 h.
5. Return to baseline health between episodes.
6. Not attributed to another disorder.
Rome IV
1. Two or more periods of intense unremitting nausea and paroxysmal vomiting, lasting hours to days within a 6-month period.
2. Episodes are stereotypical in each patient.
3. Episodes separated by weeks to months with return to baseline health between episodes.
4. Symptoms not attributed to another medical condition.

All respective criteria must be met to meet consensus definitions for both NASPGHAN and Rome IV

Inclusion of an ICD-10 code specific to CVS has enabled more complete epidemiologic surveys, documenting a 2% prevalence of adult CVS in the USA [8]. Yet, typical misdiagnoses continue today, including gastroenteritis, food poisoning, gastroesophageal reflux, and eating disorders, delaying accurate diagnosis by a median 2.6 years. In our consecutive series, CVS was second only to gastroesophageal reflux as a cause of recurrent vomiting [9]. Two school-based surveys estimated the frequency to be 2% in Scottish and Turkish children and the incidence of new cases of CVS was reported to be 3.15 per 100,000 children per year in Irish children [6, 10]. In our series, the average age of onset was 4.8 years with a predominance in girls over boys (57:43) (Table 25.2) [11].

Pathophysiologic connections have been made with mitochondrial disease, autonomic dysfunction, and the stress

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Table 25.2 Epidemiology and demographics

Features	
Age of onset	4.8 years
Delay in diagnosis	2.6 years
Prevalence	2%
Incidence	3.15/100,000
Female/male	57:43
Migraine association	39–87%

Sunku and Li [11], with kind permission from Springer Science + Business Media

response. Current research is focused on the identification of neuroendocrine mechanisms and novel therapeutic targets.

Cyclic vs. Chronic Patterns of Vomiting

An important clinical clue to the diagnosis of CVS is the pattern of vomiting. Based on the temporal pattern, children with recurrent vomiting can be delineated into cyclic and chronic groups. The *cyclic group* has an intense, but intermittent pattern of vomiting with peak emesis of at least 4/hr and minimum week-long period between episodes [12]. The *chronic group* has a low grade, daily pattern of emesis with <4 emesis/hr and >2 episodes per week [12]. Two-thirds of all children with recurrent vomiting fit into the *chronic* or continuous pattern of vomiting. These children rarely appear acutely ill or become dehydrated. Conversely, the *cyclic* pattern is associated with more intense vomiting, ill-appearance (pallor, lethargy), migraine features and these affected children more often require IV hydration compared with the chronic group (62% vs 18%) [9].

Recognizing these two patterns is also important because the groups differ significantly in symptom and diagnostic profile. In those with the cyclic vomiting pattern, nongastrointestinal disorders including neurologic (including CVS and abdominal migraine), renal, endocrine, and metabolic ones predominate over gastrointestinal disorders by a ratio of 5:1 [12, 13]. In contrast, in the chronic group, gastrointestinal disorders (mostly peptic disease) predominate over nongastrointestinal causes for vomiting by a ratio of 7:1 (Table 25.3) [12, 14]. This implies the need to center the diagnostic workup on extra-intestinal disorders in children who present with the *cyclic* vomiting pattern, while on upper GI tract disorders in the *chronic* vomiting pattern.

Clinical Patterns

CVS has a distinctive on-off temporal pattern of vomiting that serves as an essential criterion for diagnosis. CVS is distinguished by the "on" pattern of discrete, recurrent, and severe episodes of vomiting, which are stereotypical within the individual as to time of onset (usually early morning), duration (hours or days), and symptomatology (pallor, listlessness). The "off" pattern occurs during week or month-long intervals when the child resumes completely normal or baseline health (e.g., if there is other chronic disease), although 12% may have interepisodic symptoms of daily nausea and/or mild vomiting [14]. Interepisodic symptoms should always prompt workup for alternate or additional diagnoses. During the episodes, the most common symptoms are listlessness (93%) and pallor (91%) that can even mimic semicoma or shock. Other symptoms include relentless nausea, abdominal pain, low grade fever or hypothermia, intermittent flushing, diaphoresis, drooling, and diarrhea. Although found in significantly higher frequency than in patients with chronic GI disorders, fewer than half have migraine features of headache, photophobia, and phonophobia [15].

The duration of episodes generally ranges from hours to days with a median duration of 27 hours [15]. Episodes are always self-limited although a few may last longer than 1 week and require escalating medical care and nutritional support. About 49% of patients have "cyclic" or "calendar-timed" intervals predictable within a week, most commonly 4 weeks, and the remainder have "sporadic," unpredictable attacks; 42% have onset of their episodes early morning or upon awakening (1–8 am). Many have a remarkably rapid onset (median 1.5 hours) and resolution (median 6 hours) from the last emesis to the point of being able to eat and be playful. The 67% with a prodrome have pre-emesis pallor, diaphoresis, abdominal pain, and headache, but rarely the visual disturbances seen in a classical migraine aura. The prodromal phase may have therapeutic implications as there may be sufficient time to intervene medically to prevent the start of the emetic cycle.

The vomiting in CVS is uniquely rapid-fire and peaks (during the worst episode) at a median frequency of six times an hour and averages 22 times per episode. The vomiting is typically projectile and contains bile (80%), mucus, and occasionally blood, the latter usually the result of prolapse gastropathy. The bilious nature often raises concern for an obstructive lesion. The intense nausea and retching differ from that associated with emesis from gastrointestinal disorders in that it persists even after complete evacuation of gastric contents as if independent of gastric feedback. Many describe nausea as the most distressing symptom, only relieved during sleep. Due to the unrelenting nausea during episodes, these children appear much more debilitated when compared to those with gastroenteritis, often curled into a fetal position, listless, and withdrawn to the point of being unable to walk or interact. Anorexia, nausea, midline abdominal pain, and retching are the most common gastrointestinal symptoms.

Certain unusual observed behaviors during CVS episodes can raise questions about an underlying psychiatric disorder. There are children who drink compulsively and then vomit

Table 25.3 Differential diagnosis of cyclic vomiting [13]

	Chronic pattern	Cyclic pattern
Gastrointestinal	Peptic injury (esophagitis, gastritis, duodenitis)	Anatomic causes (malrotation, volvulus, duplication cyst)
	Eosinophilic esophagitis/gastroenteritis	Cholelithiasis/biliary dyskinesia
	Celiac disease	Pseudo-obstruction
	Inflammatory bowel disease	Recurrent/chronic pancreatitis
	Giardiasis	
	Chronic appendicitis	
	Gastroparesis	
Infectious	Chronic sinusitis	Sinusitis and infections may be triggers
Genitourinary	Pyelonephritis, pregnancy	Acute hydronephrosis due to ureteropelvic junction obstruction or stones
Metabolic	Rare	Mitochondrial disorders (MELAS)
		Organic acidemias
		Aminoacidurias
		Fatty acid oxidation defects
		Urea cycle defects
		Acute intermittent porphyria
Endocrine	Adrenal hyperplasia	Addison disease
		Diabetic ketoacidosis
		Pheochromocytoma
Neurological	Chiari malformation	Migraine (headaches, abdominal)
	Subtentorial neoplasm	Autonomic dysfunction
Psychiatric/ behavioral	Munchausen by proxy (rare)	Munchausen-by-proxy (rare)
	Rumination/functional vomiting	Bulimia nervosa

and describe that this maneuver dilutes the bitter bile and aids in evacuating it. Others take prolonged, hot showers or baths until the hot water supply is exhausted. This behavior has been increasingly associated with cannabinoid hyperemesis syndrome (CHS) in adults. However, current theories do not necessarily imply cannabis use in patients using hot showers for relief (as children display this without exposure) but rather that CHS patients represent a subset of CVS triggered by excessive and prolonged use [15]. Many are hyperesthetic to light, sound, motion, odor, taste, and even to parental touch and attempt to shut out all external stimuli. Nearly all turn their rooms into a darkened cave in order to avoid any stimuli that can trigger more nausea and vomiting.

Various recurring stressors have been identified by parents to precipitate CVS episodes in 76% of patients. These include psychological (44%), infectious (31%), and physical triggers. Although negative stressors are common, the psychological stress is often of an excitatory nature such as holidays, birthdays, outings, and vacations. Episodes may be triggered by various infections including upper respiratory infections, sinusitis, strep throat, and flu. The largest fraction (32%) has a seasonal clustering of episodes with more during the winter and fewer during the summer. Although this pattern correlates with the school year, we can only speculate that less school-related stress, less exposure to infections, and longer duration of sleep all factor into the improvement during summer months. Dietary triggers include foods rich in amines, aged-cheese, chocolate, monosodium glutamate, and fluctuating caffeine intake (23%). Lack of sleep from excess physical exhaustion due to travel, sports, sleepovers or a sleep disorder (24%), and menses (catamenial CVS 22%) are also common inciting events. Environmental triggers include changes in barometric pressures in weather fronts. One subgroup with a precisely timed interval every 60 days (predictable within a week) and an absence of identifiable triggers is noted to be especially refractory to therapy.

Pathophysiology

In the absence of a defined etiopathogenesis, CVS remains classified as an idiopathic disorder. Investigations support the contributory roles of mitochondrial DNA (mtDNA) mutations and dysfunction, polymorphisms involving the cannabinoid receptor type 1 and μ-opioid receptor genes (adults), dysfunction of hypothalamic-pituitary-adrenal (HPA) axis or endocannabinoid system, and autonomic nervous system (ANS) dysfunction [14]. CVS is a functional disorder of gut-brain interaction perhaps mediated through altered brainstem modulation of effector signals.

Mitochondrial Dysfunction

In two series, a striking maternal inheritance pattern was recognized for migraines in 64% and 54% of probands with CVS [16, 17]. Evidence of mitochondrial dysfunction was first provided using NMR to establish decreased ATP production in peripheral muscle in migraineurs [18]. This mitochondrial pathogenesis gained substantial support following the identification of two tandem mtDNA polymorphisms, 16,519 T and 3010A with impressive odds ratios of 17 and 15 in CVS and migraine in haplotype H, respectively [19]. Because the mutations are found in the control region rather than the enzyme sequence, the structure to function relationship is unclear. However, elevated lactate, ketones, and Krebs cycle intermediates during the early part of the attacks are consistent with mitochondrial dysfunction. In addition, clinical trials and open label experience show some effects of mitochondrial supplements Coenzyme Q10, L-carnitine, and riboflavin in the treatment of migraines and CVS in children [20, 21].

Neuroendocrine

Stressors, both psychological (excitement, panic) and physical (fever, lack of sleep), are common triggers of attacks of CVS. An activated HPA axis during episodes of CVS was first described by Wolfe and Adler and later in greater detail by Sato [22, 23]. These authors documented elevated levels of adrenocorticotropic hormone (ACTH), antidiuretic hormone, cortisol, catecholamines, and prostaglandin E_2. These elevated levels may partially explain the symptoms of hypertension and profound lethargy in this subset of patients. Attenuation of CVS symptoms occurred after use of high-dose dexamethasone by Wolfe and Adler and indomethacin and clonidine by Sato et al. [24]

These findings have focused attention upon the potential role of corticotropin-releasing factor (CRF) as a brain-gut neuroendocrine mediator of foregut motility. Taché et al. have shown that psychological or physiologic stressors induce CRF release from the hypothalamus which stimulates inhibitory motor neurons via CRF-R2 receptors in the dorsal motor nucleus of the vagus that delays gastric emptying, independent of downstream effects of ACTH, and cortisol secretion [25]. Preliminary data demonstrates increased peripheral CRF levels during episodes of CVS but whether this acts to trigger emesis or occurs in response to the stress of the illness is not clear. The new entity of cannabis-induced hyperemesis syndrome (CHS), which probably represents a variant of CVS, suggests that the endocannabinoid system plays a role in CVS. CB1 receptor activation attenuating the stress response, reducing nausea, and increasing appetite are well-known effects of THC. Interestingly, a growing number of case reports suggest that prolonged and frequent high dose use may alter the ligand-receptor relationship and result in a cannabis-triggered type of CVS [26]. This unique response suggests that in some patients, altered endocannabinoid signaling may trigger attacks. Conversely, there may be therapeutic potential in other CB1 receptor agonists.

Autonomic Dysfunction

Most of the prominent symptoms of CVS are expressed through the ANS, strongly suggesting ANS dysregulation during vomiting episodes. The peripheral vasoconstriction, hypersalivation, diaphoresis, tachycardia, and listlessness are prominent manifestations of nausea that persist throughout the episode, typically unrelieved by evacuation of the stomach. A small series of children with CVS identified autonomic dysfunction in the form of postural orthostatic tachycardia syndrome (POTS) [27]. This study noted that treatment of the POTS appeared to help reduce the frequency of CVS episodes. Further, heart rate variability studies have documented autonomic imbalance at baseline in children with CVS, manifested by diminished cardiac parasympathetic modulation [28, 29]. One small study associated state anxiety during a stress test to changes in heart rate variability in children with CVS, suggesting a possible link between anxiety and abnormal ANS reactivity serving as triggers of episodes [29]. Two formal studies of ANS function in adults with CVS revealed a pattern of heightened sympathetic tone with normal parasympathetic tone [30, 31].

We have found an overall prevalence of POTS in 19% of our CVS patients, and when we limited the cohort to adolescents >11 years in whom POTS is known to be more common, the rate was 38% [32]. This rate may be even higher in later adolescence as the disorder is frequently unrecognized and testing not widely available. Further, an evolution into chronic symptoms of ANS imbalance is noted in a subset of CVS children during teenage years. This tends to manifest by severe, daily morning nausea along with dizziness, concentration difficulties, sleep problems, and chronic fatigue. Further data is needed to characterize such subtypes.

Subtypes of CVS and Comorbidities

Migraines

An association with migraines was identified over a century ago [4, 33]. The current association occurs in 83% of those with CVS who have a positive family history of migraines or migraines themselves. In addition, there is a common progression from CVS to migraine headaches with advancing adolescent age. In the absence of definitive diagnostic tests

for migraines and CVS, strong link is further supported by similar symptomatology (e.g., pallor, lethargy, nausea, photophobia, phonophobia), positive responses in both groups to antimigraine therapy, and mtDNA polymorphisms. These migraine-associated CVS patients generally have milder episodes, a greater association with psychological stress, and significantly higher response rates to antimigraine therapy (79% vs. 36%) [34, 35].

Sumatriptan (a selective 1B/1D serotonin agonist) is one antimigraine drug that can abort episodes if administered early on especially via the nasal route (52%) [36]. This action on serotonin receptors with similar rates of response to patients with migraine headaches suggests a central role of action presumably by decreasing cerebrovascular dilatation. Until we have a clearer delineation of mechanisms involved in migraine and CVS, we cannot be certain if the CVS patients who do not fit under the migraine umbrella have dissimilar pathophysiologic cascades.

Neurocognitive Delays

In Boles' series, 25% had coexisting neurological findings of developmental delay, seizures, hypotonia, and skeletal myopathy as well as cognitive and cranial nerve dysfunction [37]. These children, classified as CVS+, were found to have an earlier age of onset for CVS and a 3- to 8-fold higher prevalence of dysautonomic (neurovascular dystrophy) and constitutional (growth retardation) manifestations than CVS patients without neurological findings.

Cannabis

As noted, there is a group of adolescents and young adults with CVS who chronically use marijuana to alleviate nausea and vomiting. However, this instead may aggravate CVS symptoms in susceptible individuals and has been labeled as CHS [15]. This more likely represents cannabis-triggered CVS [26]. Although not pathognomonic, CHS has been associated with compulsive hot water bathing [38]. CHS is now recognized in the adult Rome IV criteria as a separate entity. This is defined as cyclical emetic episodes mimicking CVS, after longstanding cannabis use, and often with associated pathologic bathing behaviors [39]. There are now over 280 case reports described, mostly young males and daily users over 6 years. One series of nine patients reports termination of bouts of emesis after cessation of chronic use of marijuana with exacerbation upon resumption of smoking cannabis, but most case reports do not provide sufficient follow-up to establish whether there is clinical resolution upon cessation of cannabis.

Other Subgroups

As discussed, some have documented ANS abnormalities and comorbid POTS in whom treatment of POTS may help reduce frequency of vomiting episodes. The Sato variant is associated with hypertension during episodes only and an endocrine profile of heightened HPA axis activation including ACTH, cortisol, cathecholamines, and vasopressin. These patients generally have significantly more prolonged episodes (102 hrs vs 50 hrs) and increased vomiting per episode (75 emesis vs 31) (Li, Unpublished data). Those with long-interval, calendar-timed episodes every 60+ days apart appear particularly difficult to treat and usually have no identifiable stress or infectious triggers. A stable periodicity has also been observed in postmenarchal girls with catemenial CVS who may respond to low-estrogen birth control pills or Depo-Provera. Finally, in a group of children, dietary triggers of CVS episodes can be identified. Some are initiated by typical migraine precipitants including cheese, chocolate, and monosodium glutamate, whereas others are triggered by other foods and/or preservatives.

It is unclear whether these groups are distinct or more likely overlapping phenotypes. We hope that delineation of clinical patterns into subgroups may ultimately point us towards more specific treatment approaches for each subgroup. New findings from mitochondrial genome studies and related nuclear genes that affect mitochondrial function may also eventually point to specific treatments.

Comorbidities

We have begun to document numerous comorbidities in affected children most of which affect them during the episode-free intervals when well. We find that they contribute to symptoms of fatigue, abdominal pain, and dizziness that impair the daily quality of life as documented by Tarbell and Li [40]. Comorbidities in non-neurologically impaired children include anxiety (47%), depression (14%) [41], chronic fatigue (52%), sleep disturbances (48%), irritable bowel syndrome (41%) [42], gastro-esophageal reflux disease (39%), colonic dysmotility (20%) [37], postural orthostatic tachycardia syndrome (19%), daily nausea (coalescent 12%), and complex regional pain syndrome (10%) [15, 43]. It is possible that several of these comorbidities are manifestations of underlying ANS dysregulation. Often, these complaints contribute to the poor quality of life with an average of 3.1 comorbid symptoms per child and have to be treated concomitantly to help restore the child to functionality.

Differential Diagnosis

Differentiating a cyclic versus a chronic pattern of vomiting is the first step in narrowing the differential diagnosis. Although the majority of patients (88%) with a cyclical pattern ultimately are diagnosed with CVS, the remaining 12% have other specific causes for vomiting found on diagnostic testing. The majority of disorders that can mimic CVS include nongastrointestinal as well as GI disorders.

Of the gastrointestinal disorders, the most serious are anatomic anomalies of the GI tract including malrotation with intermittent volvulus which can cause devastating bowel necrosis. Although not typically cyclical, we have found children with eosinophilic esophagitis related to significant food allergies to mimic CVS. Lucarelli et al. have described seven children with positive RAST to foods (milk, egg white, and soy) whose episodes diminished after specific food elimination [44].

The most common extraintestinal surgical cause is acute hydronephrosis resulting from ureteral pelvic junction obstruction. Metabolic causes include mitochondrial disorders (disorders of fatty acid oxidation, mitochondrial encephalopathy, lactic acid, and stroke-like syndrome), urea cycle defects (partial ornithine transcarbamylase deficiency), organic acidurias (proprionic acidemia), aminoacidurias, and porphyrin degradation disorders (acute intermittent porphyria) [45, 46]. Neurosurgical causes include various lesions of the subtentorial region including cerebellar medulloblastoma, brain stem glioma, and Chiari malformation, likely precipitating vomiting through increased intracranial pressure. Endocrine causes include Addison disease, pheochromocytoma, and carcinoid. Munchausen by proxy (ipecac poisoning) should also be included in the differential diagnosis of a cyclic pattern of vomiting.

Gastroparesis must be considered in the differential diagnosis. Patients with gastroparesis generally have chronic postprandial symptoms as opposed to the severe, episodic emesis of CVS within a cycle. However, a subset may have a cyclic exacerbation of upper GI tract dysmotility that can mimic CVS. Gastric emptying scans may not be helpful, as no pediatric norms are available and emptying rates may differ if assessed at baseline compared to during the prodromal or emetic episode when autonomic reactivity is high. It may also confuse the diagnosis as CVS patients are found to have various patterns of gastric emptying [47]. Instead of hypomotility, the majority of adult patients with CVS were found to have rapid gastric emptying (59%) [48], while 27% were normal and only 14% were actually delayed. This hypomotility is less pronounced in children as the respective numbers for rapid, normal, and delayed were more even [47]. This hypermotility may be a surrogate marker for autonomic dysfunction that is present in many of these patients [30, 31]. It is important to consider the limitations of these data given the absence of pediatric gastric emptying norms and the retrospective study design.

Diagnostic Evaluation

At present, there are no specific biomarkers or tests to diagnose CVS, and the diagnosis rests primarily upon fulfilling clinical criteria in the NASPGHAN Consensus Statement or Rome IV (less rigorous) [2, 3]. The key first step requires recognizing a cyclic pattern (high intensity, low frequency) of vomiting because 88% will eventually turn out to be diagnosed with CVS [9]. There are still one in eight who will have an organic underlying cause, often extra-intestinal. Underlying disorders that may require surgical intervention include anatomic anomalies of the GI tract, especially malrotation with the risk of volvulus, renal hydronephrosis, and subtentorial Chiari malformation or neoplasm. These can be excluded by upper GI series (to ligament of Treitz) renal ultrasound performed during/soon after illness (acute calyceal dilation can resolve in 10 days) and brain MRI (CT may not adequately visualize the brainstem), respectively. The challenge to the clinician is to determine which and how much testing should be performed, as the traditional 'shotgun' approach is costly, time-consuming, and invasive.

The recent NASPGHAN Consensus Statement (2008) guidelines recommend against the traditional shotgun approach and a minimal amount of screening to include an upper gastrointestinal series to exclude malrotation and anatomic obstructions and a basic metabolic profile (electrolytes, glucose, BUN, creatinine) [2]. This is supported by a recent study showing minimal yield of extensive diagnostic testing that only changes approach in 4% in pediatric CVS [49]. Similarly, our cost-decision analysis found an UGI x-ray to be the most cost-effective test followed by empiric treatment for 2 months [50]. Further testing beyond that should be based upon specific red flags or warning signs (Table 25.4). In those who present with bilious vomiting and abdominal tenderness, abdominal imaging should be performed to exclude hydronephrosis, pancreatitis, and cholecystitis. In those in whom episodes are triggered by intercurrent illnesses, fasting, or high protein meals, metabolic screening should be performed for urea cycle, fatty acid oxidation, disorders of organic and amino acid metabolism, and mitochondrial disorders. This screening has an improved diagnostic yield during the early part of an episode of CVS before intravenous glucose and fluids are administered. Those presenting with abnormal neurological findings including altered mental status, papilledema, ataxia, or sei-

Table 25.4 Evaluation of cyclic vomiting

Patient meets consensus criteria for CVS > UGI series to evaluate for malrotation + serum electrolytes, BUN, creatinine. If no warning signs or findings to suggest an organic disorder > two-month trial of empiric therapy to treat CVS.
If warning signs are present:
Severe abdominal pain, bilious, and/or hematemesis > serum chemistries, liver and pancreatic assessment, abdominal ultrasound (vs CT or MRI), esophagogastroduodenoscopy.
Fasting, high-protein meal, intercurrent illness precipitating episodes of vomiting > serum and urine metabolic evaluation (lactate, ammonia, carnitine profile, amino acids, and organic acids) *prior to intravenous fluid treatment during episode* as well as metabolic consult.
Abnormal neurological findings (altered mental status, papilledema) > brain MRI, neurology consult.

Table 25.5 Rescue and abortive pharmacotherapy

Antimigraine
Sumatriptan 20 mg intranasal at episode onset and may repeat once vs. 25 mg po once vs. 3–6 mg s.c. once SE: Chest and neck burning, coronary vasospasm, headache
Alternatives: *Rizatriptan*, *zolmitriptan, frovatriptan (longer half-life)*
Antiemetic
Ondansetron 0.2–0.3 mg/kg per dose (≤12 mg) q 4–6 h iv/po/rectal/topical. SE: Headache, drowsiness, dry mouth
Alternatives: *Granisetron*
Aprepitant 3 day regimen: 125, 80, 80 mg one q.d. prior to anticipated episode
Fosprepitant 3–4 mg/kg (max 150 mg) IV day one (aprepitant days 2–3)
Sedative
Lorazepam 0.05–0.1 mg/kg per dose q 6 h iv/po: Useful adjunct to ondansetron. SE: Sedation, respiratory depression
Chlorpromazine 0.5–1 mg/kg per dose q 6 h iv/po. SE: Drowsiness, hypotension, seizures, dystonic reaction
Diphenhydramine 1.25 mg/kg per dose q 6 h iv/po: Useful adjunct to chlorpromazine. SE: Hypotension, sedation, dizziness
Dexmedetomidine bolus 0.5mcg/kg over 15 min > 0.5mcg/kg/h (up to 1.5 mcg/kg/h) continuous infusion
Analgesic
Ketorolac 0.5–1 mg/kg per dose q 6 h iv/po. SE: Gastrointestinal bleeding, dyspepsia

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SE side effects

zure should have a neurological evaluation and brain MRI. Presentation of CVS under the age of 2 should also prompt further metabolic or neurological testing [2].

Treatment

Current treatment for CVS can be divided into supportive or rescue therapy (during episodes), lifestyle modifications and prophylactic (daily treatment to prevent episodes), and abortive therapy (prodromal intervention to abort episodes). The goals of treatment are to reduce the frequency and severity of episodes, enhance functionality, and improve quality of life. Treatment of nausea, vomiting, abdominal pain, and dehydration during acute episodes requires a protocol for use at home, in emergency departments, and on hospital wards. Other strategies for management of CVS include avoidance of identified triggers (e.g., dietary cheese), psychological interventions (e.g., stress management), and treatment of comorbid symptoms.

The NASPGHAN Consensus Statement recommendations on treatment are based upon therapeutic responses from case series and expert opinion of the task force [2]. The main recommendations include first line prophylactic use of cyproheptadine and amitriptyline in children under and over age 5 years, respectively, with propranolol serving as the second line. Sumatriptan was recommended as an abortive agent for those >12 years. For rescue therapy during acute episodes, IV rehydration with higher dose antiemetic ondansetron (0.3–0.4 mg/kg/dose) and sedation from diphenhydramine or lorazepam was recommended.

Supportive or Rescue Therapy

Supportive or rescue care is used when the vomiting becomes well established in an episode and at that point usually fails to respond to any abortive strategies. The goal is to correct energy, fluid, and electrolyte deficits and render the child more comfortable through antiemetic therapy, analgesics, and sedation for relief from intractable nausea, vomiting, and abdominal pain. The recommendation is for an IV bolus of saline for rapid correction of fluid deficits and concurrent 10% dextrose 0.45 normal saline at 1.5 X maintenance rates to provide sufficient cellular energy to terminate ketosis. One may have to reduce IV rates and increase Na^+ content when hyponatremia and diminished urine output ensues from elevated antidiuretic hormone release present in Sato-variant CVS. Ondansetron has been the most widely used $5HT_3$ antagonist given safely at higher than standard doses (up to 0.3 mg/kg/dose) but can prolong the QTc interval [16]. It generally reduces both nausea and vomiting but usually does not stop the episode or the misery from nausea (Table 25.5). A recent systematic review found greatest evidence for ondansetron as rescue therapy in the emergency department for pediatric CVS with promising support for sumatriptan and aprepitant [51].

Diphenhydramine, lorazepam, diazepam, or chlorpromazine combined with diphenhydramine is used to induce sedation because sedation is often the only means of providing relief from the unrelenting nausea and abdominal pain. The analgesic ketorolac is recommended as first line as narcotics should be avoided and are felt to have a sensitizing effect in migraine analgesia. A nonstimulating environment including quiet, dark single room may be helpful. When all else fails

and episodes are prolonged and debilitating (>1 week), we have occasionally used a dexmedetomidine infusion to achieve deep sedation in a PICU setting for 18 hours as described by Khasinwah [52].

Lifestyle Modifications

Lifestyle modifications are used during the interictal phase of CVS when the child is not in an episode in order to keep the child properly conditioned and to avoid exposure to known and potential precipitants of episodes. The lack of sleep resulting from disturbed sleep patterns, sleepovers, or travel sports tournaments are often cited as triggers of episodes. Good sleep hygiene (e.g., turning off all phones, computers, music, TV) with a regimented sleep time can reduce the frequency of episodes. Providing at least maintenance volumes of fluids is widely used to prevent migraines and postural orthostatic tachycardia syndrome. Providing low glycemic energy sources before strenuous activity, sources may prevent an exercise-induced energy deficit. Routine exercise can help reverse the deconditioned state and improve mitochondrial function. Finally, avoiding identified triggers specific to the individual (e.g., sleepovers) or including those generally found in migraines (monosodium glutamate and fluctuations in caffeine intake) may help reduce the frequency of episodes. Fleisher reported that consultation and lifestyle modifications alone reduced the frequency of episodes in 70% of patients even before beginning standard prophylactic therapy [42].

Prophylactic Therapy

For those with more frequent or severe episodes (e.g., more than once a month), prophylactic therapy daily (or twice weekly with aprepitant) is recommended during the interictal phase with the goal of preventing the next episode or to at least reduce the frequency, duration, or intensity (# emeses) of episodes. These *prophylactic medications* are traditionally used to treat other disorders including migraines. The NASPGHAN consensus recommendations for the initial treatment were for cyproheptadine for the younger (<5 years) and amitriptyline for the older children and adolescents (≥5 years) (Table 25.6) [2]. Despite its pharmacokinetics, cyproheptadine (0.25–0.5 mg/kg) appears to be effective given as a single night time dose as opposed to the usual two or three divided doses [53]. Amitriptyline causes side effects in 50%, the most common being morning sedation (like a hangover), and is stopped in 21% [54]. Beginning at a low dose of 0.2 to 0.3 mg/kg at bedtime and titrating it gradually in 10 mg increments every week or two to the target dose of 1.0–1.5 mg/kg allows the child to gradually adapt to the side effects. Switching to other tricyclic antidepressants such as nortriptyline, desipramine, or doxepin may lessen specific side effects. An EKG to measure the QTc interval is recommended before starting amitriptyline. At higher doses (>1.0 mg), we monitor the ECG and/or amitriptyline levels [55]. Propranolol is second line therapy and can be monitored for efficacy and toxicity by a desired drop in pulse rate of 15–20 beats per minute not to fall below 55 bpm, respectively.

Table 25.6 Prophylactic pharmacotherapy

Antimigraine
Amitriptyline start and 0.2–0.3 mg/kg and advance to 1–1.5 mg/kg/day q.h.s.: Monitor EKG QTc interval prior to starting and with higher dose titrations. First choice ≥5 years old. Side effects: Sedation, anticholinergic
Propranolol 0.25–1 mg/kg/day divided b.i.d or t.i.d: Monitor resting heart rate. SE: Hypotension, bradycardia, fatigue
Cyproheptadine 0.25–0.5 mg/kg/day divided b.i.d. or q.h.s.: First choice <5 years old. SE: Sedation, weight gain, anticholinergic
Alternatives: *Nortriptyline*, *desipramine, doxepin*
Anticonvulsants
Topiramate titrate to 1.5–2.0 mg/kg/day divided b.i.d. SE: Appetite suppression, cognitive dysfunction, renal stones
Phenobarbital 2–3 mg/kg/day q.h.s. SE: Sedation, cognitive impairment
Alternatives: *Gabapentin, levetiracetam, zonisamide, valproate, carbamazepine*
NK-1 receptor antagonist
Aprepitant 125 mg PO twice weekly (>60 kg); 80 mg (40–60 kg); 40 mg (<40 kg)
Mitochondrial supplements
l-*carnitine* 50–100 mg/kg ≤ 2 g/day divided b.i.d. SE: Diarrhea, fishy body odor
Coenzyme Q_{10} 10 mg/kg/divided b.i.d. ≤600 mg/day
Riboflavin 10 mg/kg/day divided b.i.d. ≤400 mg/day

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If standard prophylactic therapy fails, aprepitant, anticonvulsants, and Ca^{++}-channel antagonists have been used. In a series of children and adolescents, 81% receiving Aprepitant achieved a partial (62%) or complete (19%) response when administered twice weekly [56]. Topiramate (1–2 mg/kg/day) and phenobarbital at low (2–3 mg/kg) nighttime doses have been reported to be effective, the former having a strong evidence based in migraines [57, 58]. Unfortunately, cognitive dysfunction is a well-known side effect of both. A retrospective review of 38 pediatric CVS patients documented efficacy in 81% of patients treated with topiramate vs 59% treated with propranolol [59]. A recent randomized trial from Iran found amitriptyline (1 mg/kg/d) superior to topiramate (1–2 mg/kg/d) in 70 children with CVS, documenting short-term efficacy in 68% vs. 39% of patients, respectively [60]. Other effective anticonvulsants, zonisamide and levitiracetam, have been effective in adults with CVS [61]. Another group of agents includes Ca^{++}-channel antagonists with the

main side effect of hypotension. Other antidepressants may also be considered when there is strong psychiatric comorbidity. A pediatric case series documented efficacy of the tetracyclic antidepressant mirtazapine in CVS patients with anxiety and depression [62]. Mirtazapine also facilitates both sleep and appetite and may thus be considered in specific patients with sleep disturbances and poor weight gain.

Treatment may vary by clinical subgroup. Children that have a migraine connection with either a positive family history or migraines themselves are much more likely to respond to antimigraine agents such as cyproheptadine, amitriptyline, and propranolol (79 vs. 36%) than those children without one [34]. Postmenarchal girls with catamenial CVS often respond to low estrogen birth control pills or Depo-Provera. Sato-variant CVS associated with hypertension may require intra-episodic short-acting antihypertensives as well as prophylactic tricyclic antidepressants in the USA and divalproex sodium in Japan [23].

Prophylactic use of mitochondrial supplements as adjunctive prophylactic therapy in CVS is quite common based upon evidence in migraines and preliminary evidence in children with CVS. These supplements have demonstrated efficacy in the prevention of migraines in adults (randomized controlled trials) and preliminary evidence of efficacy in pediatric migraine and CVS in children [54, 63–65]. Interestingly, in some children with CVS, the accompanying chronic fatigue may respond to these supplements. The doses used include CoQ10 10 mg/kg divided b.i.d. up to 600 mg/day, riboflavin at 10 mg/kg divided b.i.d. to up to 400 mg/day, and L-carnitine at 50–75 mg/kg up to 3 g/day divided b.i.d. The dose and duration of these supplements has not been established in children with CVS.

Abortive Therapy

Abortive therapy should be considered for those who have sporadic or milder episodes that occur less than once per month and who prefer not taking prophylaxis or those who have breakthrough episodes while on prophylaxis. Abortive therapy is administered during the prodrome or at the beginning of the vomiting episode in the hope of stopping it. The novel NK-1 receptor antagonist aprepitant is a highly effective antiemetic when administered as an abortive agent in the prodromal phase at least 30 min before vomiting begins (only oral formulations available). This may be particularly effective in calendar-timed CVS if administrated prior (e.g., a day before) to the anticipated episode. One retrospective pediatric study documented efficacy of aprepitant 3-day regimen for abortive use (Table 25.5) [56]. Subtype-specific abortive therapy includes antimigraine triptans for those with migraine features. The nasal (sumatriptan or zolmitriptan) or subcutaneous (sumatriptan) forms appear more effective than oral forms that cannot be effectively absorbed due to repeated vomiting (Table 25.6) [2, 66]. The triptans are usually either fully effective or not at all and more effective if administered early in episode or if the duration of episodes is less than 24 hours (Li, unpublished data).

In a few children, ondansetron administered alone aborts episodes in progress. Although the oral forms may not reach the duodenum, ondansetron can be reformulated by individual pharmacies into a rectal suppository or topical (lipodermal) forms. Although not established, we successfully use the same dosages as the oral form.

Comorbidities

Treatment of specific comorbid conditions and symptoms may alleviate the CVS, improve quality of life, and reduce functional disability. Most of important of these is addressing anxiety through stress reduction techniques (e.g., cognitive behavioral therapy) as well as anxiolytics. This approach has anecdotally reduced the number of episodes in anxious children whose attacks are triggered by stressors and/or panic anxiety. Chelimsky has shown in a small series that treatment of the POTS with fluids, salt, fludrocortisone, and/or propranolol helps prevent attacks of CVS [27]. Alleviating other comorbid symptoms – disordered sleep, chronic fatigue, and irritable bowel syndrome – may substantially improve the quality of life and functional disability.

Natural History, QOL Impact, and Complications

There is some data for the natural history of CVS. Our projection analysis estimates that 75% of patients will develop migraine headaches by age 18 (Li Unpublished data). Other long-term studies have shown that up to half of CVS patients continue with CVS or migraine headaches. Several studies have noted the mean duration of illness to be around 6 years, but in our cohort, the younger the age of onset, the longer the duration. Also 5% of patients will progress through all 3 phases of periodic disease from CVS to abdominal migraine and finally to migraine headaches. We are currently encountering a growing group with teenage-onset CVS and it is too early to ascertain if they are at greater risk of carrying CVS into adulthood.

CVS has a significant deleterious impact on the quality of life in affected children. Although well in between episodes approximately 90% of the time, 58% of affected children require intravenous fluids during episodes and average 10 visits to the Emergency Department [67]. School-age children in our cohort missed an average of 24 days of school per year [13]. Medical morbidity is reflected by the high average

annualized total cost of $17,035 in 1999 dollars that includes doctor visits, emergency department visits, inpatient hospitalizations, missed work by parents, as well as biochemical, radiographic, and endoscopic testing [50]. A more recent pediatric study documented a mean cost of screening diagnostic workup per CVS patient at $6125 [49]. This study found that most (96%) patients did not undergo any change in diagnosis or medical management based on the laboratory evaluation. Growing number of comorbid conditions such as anxiety and postural orthostatic tachycardia syndrome also contribute to functional impairment. We have demonstrated significantly lower quality of life scores than in healthy controls, equivalent to those in children with organic GI diseases (e.g., inflammatory bowel disease, gastritis) [40].

Complications and medical morbidity include iatrogenic tests and interventions from the misdiagnoses that are often applied to recurrent vomiting. Most are mislabeled as acute gastroenteritis, gastro-esophageal reflux, and food poisoning and treated in urgent care settings. Some with severe pain, bilious vomiting, and intractability have undergone unnecessary laparotomy, appendectomy, cholecystectomy, and Nissen fundoplication. Others have been labeled with psychiatric disorders including bulimia and psychogenic vomiting and hospitalized on psychiatric wards, while a few parents have been referred for suspicion of Munchausen-by-proxy.

Medical complications can also occur from the frequent and often severe episodes of vomiting that occur with CVS. Dehydration and electrolyte disturbances are common and IV rehydration is required at some point in 58% of patients, which can be compared to less than 1% in rotavirus. Hematemesis can occur towards the end of attacks and is usually related to prolapse gastropathy or Mallory-Weiss tears. Although not common, frequent vomiting can lead to secondary peptic injury.

Summary

CVS is a disorder that now well described and accepted in the literature and increasingly diagnosed by pediatric and family physicians as well as pediatric and adult emergency medicine specialists, neurologists, and gastroenterologists. Recently, there is greater appreciation of comorbid conditions and symptoms that deleteriously affect quality of life and functioning. Although the precise pathophysiology remains unknown, we have growing evidence of involvement of the autonomic nervous system, stress axis, and mitochondrial function. Unfortunately, no robust treatment trials have been completed to date and so therapies remains empiric. Yet, the progress made over the past two decades has been impressive.

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Food Allergy

26

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Introduction

An expert panel convened by the National Institute of Allergy and Infectious Diseases defined food allergy as "an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food" [1]. This description distinguishes food allergy from nonimmune adverse reactions to foods (Table 26.1). Over 170 food allergens have been identified, but nine foods or food groups (milk, egg, peanut, tree nuts, soy, wheat, fish, crustacean shellfish, and sesame) account for 90% of significant allergic reactions [1, 2]. It is important to note that parental perception of food allergy is generally much higher than physician-diagnosed food allergy, particularly in early childhood. Up to one-third of parents report at least one adverse reaction to food [3–8]. The emotional impact of food allergy is considerable, with significant impact on health-related quality of life [9, 10]. This chapter reviews the spectrum of food-allergic disorders with an emphasis on those relevant to the pediatric gastroenterologist. Celiac disease is the result of an immune response to foods, but is not typically categorized as an allergy and is reviewed in a separate chapter.

Epidemiology

The Centers for Disease Control and Prevention reported that the prevalence of food allergy in US children increased from 3.4% in 1997–1999 to 5.1% in 2009–2011 [11]. A study by Gupta et al. examining data from 2009 to 2010 estimated the prevalence of childhood food allergy in US children to be up to 8% [1, 12–15]. Most recently, the same group conducted a population-based survey between October 2015 and September 2016 to reassess the prevalence of food allergy in US children. The study found the prevalence to be 7.6% after excluding 4% of children whose parent-reported food allergy reaction history was inconsistent with IgE-mediated food allergy [16, 17]. The most recent study used more stringent criteria to define "convincing" allergies, thus precluding direct comparison of the estimates. The most prevalent allergens were peanut (2.2%), milk (1.9%), shellfish (1.3%), and tree nut (1.2%) [16]. Among food-allergic children, 42.3% reported ≥1 severe food allergy and 39.9% reported multiple food allergies. Furthermore, 19.0% reported ≥1 food allergy-related emergency department visit in the previous year and 42.0% reported ≥1 lifetime food allergy-related emergency department visit. Prevalence rates of food allergy were higher among African American children and children with atopic comorbidities [16].

A population-based survey study from 2015 to 2016 of US adults found that an estimated 10.8% had a convincing food allergy, confirming that many children do not outgrow their food allergies [18]. Among food-allergic adults, 51.1% experienced a severe food allergic reaction, 45.3% were allergic to multiple foods, and 48.0% developed food allergies as an adult [18]. The most common allergies were shellfish (2.9%), milk (1.9%), peanut (1.8%), tree nut (1.2%), and fin fish (0.9%) [18].

Adverse Food Reactions

Adverse food reaction is a broad term that describes an abnormal response of the body to ingestion of a particular food. This encompasses both immune-mediated and nonimmune-mediated processes.

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Food allergy is defined as an adverse specific immune response that occurs reproducibly on exposure to a given food. On the other hand, *food intolerance* is a type of nonallergic adverse reaction, defined as difficulty digesting or metabolizing a particular food. Food intolerance can arise from properties of the food itself (e.g., toxic contaminants or pharmacologic properties) or host characteristics (e.g., metabolic disorders that lead to poor digestion of food). Some well-defined examples of food intolerances are listed in Table 26.1.

Distinguishing true food allergy from food intolerance is important. In general, food intolerance presents with prominent gastrointestinal symptoms, symptom severity correlates with the amount of food ingested, and each exposure results in similar symptoms. On the other hand, IgE-mediated food allergy reactions are less predictable, and even small amounts of food have the potential to cause severe or life-threatening reactions.

Table 26.1 Nonimmune-mediated reactions or food intolerance/adverse reactions to food

Type	Example(s)
Metabolic	Lactose intolerance, galactosemia, alcohol dehydrogenase deficiency
Pharmacologic	Caffeine (jitteriness), tyramine in aged cheeses (migraine), alcohol, histamine
Toxic	Bacterial food poisoning, scombroid fish poisoning (histamine)
Undefined mechanisms	Reactions to sulfites, tartrazine

Immunopathogenesis and Specific Disorders

Tolerance is the normal immune response to food [19]. The immune system recognizes food proteins, and IgG and IgA antibodies may be generated, but there are no adverse reactions. Food allergy results from a disruption of normal tolerance mechanisms. Food allergies may be immunoglobulin E (IgE)-mediated, cell-mediated, or "mixed" adverse immune responses. The distinction of pathophysiology is important in diagnosis and management. For example, IgE-mediated reactions are typically sudden in onset following exposure to a food allergen, whereas cell-mediated responses may result in chronic inflammation or delayed symptoms. Table 26.2 highlights gastrointestinal manifestations of food allergy according to pathophysiology.

IgE-Mediated Reactions

Natural History of IgE-Mediated Reactions

IgE-mediated food allergy often appears during the first 2 years of a child's life. The sensitivity to some allergenic foods (egg, milk) self-resolves in 85% of children during childhood [20]. Sensitivity to peanuts, tree nuts, fin fish, and shellfish persists into adulthood in the large majority of affected children. Approximately, 20% of peanut-allergic children under the age of 2 years and 10% of children with tree nut allergy will become tolerant to those foods by the time they are of school age [21]. Though shellfish, tree nut,

Table 26.2 Gastrointestinal food allergies

Disorder	Mechanism	Symptoms	Diagnosis
Pollen-food allergy syndrome (oral allergy syndrome)	IgE mediated	Mild pruritus, tingling, and/or angioedema of the lips, tongue, or oropharynx	Clinical history and positive sensitization to pollens
Gastrointestinal "anaphylaxis"	IgE mediated	Rapid onset of nausea, abdominal pain, cramps, vomiting, and/or diarrhea; other target organ responses (i.e., skin, respiratory tract) often involved	Clinical history and positive tests for food-specific IgE; ± oral challenge
Eosinophilic esophagitis	IgE mediated and/or cell mediated	Gastroesophageal reflux or excessive spitting-up or emesis, dysphagia, intermittent abdominal pain, irritability, sleep disturbance, failure to respond to conventional reflux medications	Clinical history, endoscopy and biopsy, elimination diet, and oral food challenge (possible test directed)
Eosinophilic gastroenteritis	IgE mediated and/or cell mediated	Recurrent abdominal pain, irritability, early satiety, intermittent vomiting, FTT and/or weight loss, peripheral blood eosinophilia (in 50%)	Clinical history, endoscopy and biopsy, elimination diet, and challenge (possibly test directed)
Food-protein-induced proctocolitis	Cell mediated	Gross or occult blood in stool; typically thriving; usually presents in the first few months of life	Negative SPT responses; elimination of food protein → clearing of most bleeding in 72 h; ± endoscopy and biopsy; challenge induces bleeding within 72 h
Food-protein-induced enterocolitis syndrome	Cell mediated	See Tables 26.6 and 26.7	History, response to elimination/oral food challenge
Food-protein-induced enteropathy	Cell mediated	Diarrhea or steatorrhea, abdominal distention and flatulence, weight loss, FTT, ± nausea and vomiting	Endoscopy and biopsy

FTT failure to thrive, *SPT* skin prick testing, *IgE* immunoglobulin E

and fin fish allergies were the most common adult-onset food allergies, adult-onset food allergies to all major food allergen groups is also possible [22].

Symptoms

Symptoms triggered by IgE-mediated reactions occur rapidly following ingestion of the trigger food. IgE antibodies that are specific for regions (epitopes) of food proteins bind to tissue mast cells and circulating basophils. Cross-linking of the IgE antibodies by the food proteins leads to release of preformed mediators (such as histamine and platelet-activating factor) from cytoplasmic granules and transcription of inflammatory cytokines. The target organs(s) involved in the reaction define the type of food allergy. The symptoms occurring during an acute reaction can be classified as cutaneous, ocular, gastrointestinal, respiratory, cardiovascular, or neurologic. A combination of these symptoms may occur.

Cutaneous manifestations of an acute food allergy reaction include erythema, hives, pruritus, flaring of eczematous lesions, and angioedema. Food allergy may account for up to 20% of new-onset urticaria [23, 24]. Food allergies rarely cause chronic urticaria (e.g., episodes occurring regularly for 6 weeks or longer). Eczema (atopic dermatitis) can be chronically exacerbated by specific IgE-mediated food allergy, with improvement upon removal of the suspect food [25–27]. Overall, skin symptoms are the most common manifestation of IgE-mediated food allergies. Food can also induce skin symptoms by direct skin contact (contact urticaria) [28–32].

Ocular symptoms include pruritus, tearing, conjunctival erythema, and periorbital edema.

Gastrointestinal symptoms include nausea, vomiting, diarrhea, and abdominal pain. Isolated acute gastrointestinal reactions are uncommon. In the case of a food-allergic reaction, upper gastrointestinal symptoms usually begin within minutes of ingestion, but may take as long as 2 h to develop. Diarrhea may have a more delayed onset, beginning 2–6 h after ingestion of the allergen.

Respiratory tract symptoms may be acutely induced by IgE-mediated reactions. Symptoms may include pruritus and edema of the larynx, dyspnea, nasal congestion, rhinorrhea, hoarseness, stridor, tachypnea, wheezing, and cough.

Cardiovascular symptoms associated with acute food-allergic reactions include increased vascular permeability, widened pulse pressure, increased heart rate and cardiac output, and flushing. These effects can lead to the decreased organ perfusion that is characteristic of anaphylactic shock.

Neurologic manifestations include a sense of impending doom, dizziness, confusion, incontinence, and loss of consciousness. Neurologic compromise is thought to result from hypotension and hypoxia [33].

Anaphylaxis

Anaphylaxis is traditionally defined as a systemic, rapid-onset, severe, and potentially life-threatening allergic reaction resulting from the sudden release of mast cell mediators. Diagnosis of anaphylaxis is critical as these patients need prompt administration of epinephrine; however, it is not always easy to recognize. Symptoms generally include a combination of the above symptoms (or respiratory or cardiac symptoms alone), with anaphylactic shock referring to signs of poor organ perfusion in addition to anaphylaxis. In children, respiratory, cutaneous, and gastrointestinal symptoms are prominent, and the most common cause of death is respiratory compromise.

A clinical diagnostic criteria for anaphylaxis developed by a multidisciplinary group of experts (Table 26.3) helps clinicians recognize the variability in presentation [34]. This criteria has a sensitivity of >95%. It is also important to recognize that although anaphylaxis represents the most severe form of allergic reaction, there is a spectrum of severity in anaphylaxis itself. Anaphylaxis can range from mild or transient symptoms to hemodynamic compromise/shock. Less than 1% of anaphylaxis cases (including those not due to food ingestion) result in death, indicating fatal anaphylaxis is

Table 26.3 Diagnostic criteria for anaphylaxis

Anaphylaxis is highly likely when any ONE of the following three criteria is fulfilled:
1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula) AND AT LEAST ONE OF THE FOLLOWING: A. Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, hypoxemia) B. Reduced BP* or associated symptoms of end-organ dysfunction (e.g., hypotonia, collapse, syncope, incontinence)
2. TWO OR MORE OF THE FOLLOWING that occur rapidly after exposure to a LIKELY allergen for that patient (minutes to several hours): A. Involvement of the skin mucosal tissue (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula) B. Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, hypoxemia) C. Reduced BP* or associated symptoms (e.g., hypotonia, collapse, syncope, incontinence) D. Persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting)
3. Reduced BP* after exposure to a KNOWN allergen for that patient (minutes to several hours): A. Infants and children—Low systolic BP (age-specific)* or greater than 30% decrease in systolic BP B. Adults—Systolic BP of less than 90 mmHg or greater than 30% decrease from that person's baseline
BP: blood pressure. * Low systolic blood pressure for children is defined as: Less than 70 mmHg from 1 month to 1 year Less than (70 mmHg + [2 × age]) from 1 to 10 years Less than 90 mmHg from 11 to 17 years

Reproduced from: Sampson et al. [34]

rare [33–35]. Several grading systems have been used to define reaction severity (Tables 26.4 and 26.5).

In retrospective analyses, several factors appear to be associated with the severity of the allergic response. A larger quantity of food allergen ingested, concomitant alcohol consumption, fever, and concomitant nonsteroidal anti-inflammatory drugs (NSAID) use all appear to increase the rapidity and severity of the reaction [35–39]. Concomitant ingestion of fatty foods appears to slow the rate of absorption and thus delay onset of symptoms.

Risk-taking behaviors among adolescents and young adults, including increased incidents of exposure to the avoided allergen and a lack of a prompt treatment response to symptoms, contribute to the disproportionately higher number of fatal food-induced anaphylaxis in this age group [40]. In one case series of fatal food-induced anaphylactic reactions, accidental ingestion of a known food allergen was present in 87% of cases [35]. In several cases, previous reactions to the known allergen were relatively mild, highlighting the inconsistency in severity of reactions. Having asthma is an additional risk factor for fatal anaphylaxis.

Anaphylaxis is usually characterized by rapid onset and evolution of symptoms, but the time course can be unpredictable. Biphasic anaphylactic reactions occur in up to 20% of cases [41]. In this scenario, a second wave of symptoms occurs 1–4 hours following resolution of the initial anaphylactic reaction. Delayed anaphylaxis is rare and refers to anaphylaxis characterized by a delayed onset of symptoms, from minutes or even hours. Protracted anaphylaxis is an allergic reaction that can last hours or even days [42].

Table 26.4 Criteria for severity grading (Muraro et al., 2007) staging system of severity of anaphylaxis

Stage	Defined By
1. Mild (skin and subcutaneous tissues, GI, and/or mild respiratory)	Flushing, urticaria, periorbital or facial angioedema; mild dyspnea, wheeze or upper respiratory symptoms; mild abdominal pain and/or emesis
2. Moderate (mild symptoms + features suggesting moderate respiratory, cardiovascular or GI symptoms)	Marked dysphagia, hoarseness and/or stridor; shortness of breath, wheezing and retractions; crampy abdominal pain, recurrent vomiting and/or diarrhea; and/or mild dizziness
3. Severe (hypoxia, hypotension, or neurological compromise)	Cyanosis or $SpO_2 < 92\%$ at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence

Reproduced from Muraro et al. [33]

Food-Dependent, Exercise Induced Anaphylaxis

Food-dependent, exercise-induced anaphylaxis is an IgE-mediated food-induced anaphylactic reaction that occurs when vigorous exercise is performed within a few hours of food allergen ingestion. Neither exercise alone nor ingestion of the food allergen alone is sufficient to cause symptoms. While generally associated with specific causative foods (such as wheat, other grains, celery, seafood, or nuts), in some cases any food can cause the reaction when consumed in close temporal relation to exercise [44].

Alpha-Gal

Another syndrome of anaphylaxis is delayed anaphylaxis occurring several hours after ingestion of mammalian meat or "red meat" including pork, beef, and lamb. This is attributed to an IgE response against a carbohydrate moiety, galactose-α-1,3-galactose (α-gal). Unlike other food allergies, which typically occur within minutes of ingestion,

Table 26.5 The CoFAR grading system for allergic reactions

Grade 1 – Mild	Grade 2 – Moderate	Grade 3 – Severe	Grade 4 - Life Threatening	Grade 5 – Death
Transient or mild discomforts (<48 hrs), no or minimal medical intervention/therapy required. These symptoms may include pruritus, swelling or rash, abdominal discomfort or other transient symptoms.	Symptoms that produce mild to moderate limitation in activity some assistance may be needed; no or minimal intervention/therapy is required. Hospitalization is possible. These symptoms may include persistent hives, wheezing without dyspnea, abdominal discomfort/ increased vomiting or other symptoms	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible Symptoms may include Bronchospasm with dyspnea, severe abdominal pain, throat tightness with hoarseness, transient hypotension among others. Parenteral medication(s) are usually indicated.	Extreme limitation in activity, significant assistance required; significant medical/ therapy. Intervention is required; hospitalization is probable. Symptoms may include persistent hypotension and/or hypoxia with resultant decreased level of consciousness associated with collapse and/or incontinence or other life threatening symptoms.	Death

Reproduced from Burks et al. [43]

symptoms from eating red meat may be delayed, occurring 3–8 hours after eating [45]. Most food allergies are directed against a protein molecule, but alpha-gal is unusual because it is a carbohydrate, and a delay in its absorption may explain the delay in symptoms. The etiology is presumed to be related to exposure to this allergen via tick bites [46–49]. Alpha-gal is a molecule carried in the saliva of the Lone Star tick and other potential arthropods typically after feeding on mammalian blood. People that are bitten by the tick, especially those that are bitten repeatedly, are at risk of becoming sensitized and producing the IgE necessary to then cause allergic reactions. As might be expected, the incidence of tick bites is much higher in the southern and eastern United States, the traditional habitat for the tick. However, cases are now increasingly reported in the northern and western states. This phenomenon has also been observed worldwide, with different ticks responsible for similar cases of red meat allergy in many other countries including Sweden, South Africa, and Australia [50]. Immediate symptoms such as hives or shortness of breath are treated with antihistamines and epinephrine depending on the severity of the reaction. Prevention long-term involves avoidance of all red meat in sensitized individuals. Of note, poultry and fish can still be consumed without reaction.

Pollen Food Allergy Syndrome (Oral Allergy Syndrome)

Pollen food allergy syndrome is a relatively common form of food allergy that occurs in people who have pollen allergy, although not all patients have obvious seasonal allergy symptoms. Sensitization initially occurs from inhalation of pollens, but results in symptoms when fresh fruits or vegetables with proteins that are homologous to the pollens are ingested. The symptoms are IgE-mediated and result from contact urticaria in the oropharynx caused by pollen-related proteins in these foods. For example, birch pollen contains proteins homologous with Rosaceae fruits (e.g., apple, peach, carrot). Pruritus and mild swelling of the lips, tongue, and throat occur when specific uncooked fruits and vegetables are ingested, but heated forms are tolerated [48]. Symptoms typically last several minutes before self-resolving. While anaphylaxis associated with oral allergy has been reported, it is relatively rare [49]. This type of allergy has also been called oral allergy syndrome (OAS); however, OAS has been imprecisely applied in the literature to describe oropharyngeal reactions due to a variety of non-plant foods as well as both oropharyngeal and systemic symptoms due to plant foods in subjects with pollen allergy. Therefore, pollen food allergy syndrome (PFAS) is now increasingly used and the preferred term both to emphasize the pathogenesis of these reactions, and to describe the full range of oropharyngeal and systemic symptoms that can occur in response to pollen-related foods [1].

Management of Acute IgE-Mediated Reactions

Patients with food allergy should have an up-to-date food allergy action plan to guideline emergency treatment. Mild IgE-mediated reactions that only involve one organ system may subside with antihistamines. However, progressive, multisystem, or severe reactions should be treated promptly with intramuscular epinephrine. Given the inability to predict severe reactions and evidence that early epinephrine may help reduce risk, all anaphylactic reactions (irrespective of severity) demand appropriate treatment with intramuscular epinephrine [51]. Severe reactions may require multiple doses of epinephrine, intravenous fluids, oxygen, vasopressors, additional treatments, and monitoring in an acute care setting. Oral corticosteroids may prevent development of a biphasic reaction, although data is limited.

Cell-Mediated Food-Allergic Disorders

Cell-mediated food-allergic reactions include food-induced pulmonary hemosiderosis, food-protein-induced enterocolitis syndrome (FPIES), food-protein-induced enteropathy, food-protein-induced proctitis and proctocolitis, and celiac disease. Celiac disease is discussed in Chap. 40.

Pulmonary hemosiderosis is a rare condition characterized by pulmonary infiltrates, iron-deficiency anemia, hemosiderosis, hemoptysis, cough, wheezing, nasal congestion, recurrent otitis media, dyspnea, colic, diarrhea, vomiting, hematochezia, and failure to thrive attributed to cow's milk. While the immunologic mechanisms underlying this illness are not understood, peripheral eosinophilia and IgG to cow's milk are generally present. Symptoms remit with elimination of the causative allergen [52, 53].

Non-IgE-mediated gastrointestinal disorders are highlighted as follows.

Food Protein Induced Enterocolitis Syndrome

Background

Food-protein-induced enterocolitis syndrome (FPIES) is a cell-mediated gastrointestinal food allergy that typically manifests in infancy and often resolves by 3 years of age, although current evidence indicates that only 20% of children with FPIES to cow's milk and soy resolved by 3 years of age. In the acute form, 1–3 h after ingestion of the caus-

Table 26.6 Food-protein-induced enterocolitis syndrome (FPIES)

Clinical findings	*Acute:*
	Profuse, repetitive vomiting occurring 1–3 h after food ingestion
	Diarrhea occurring 5–8 h after ingestion
	Dehydration
	Lethargy
	Pallor
	Abdominal distention
	Bloody diarrhea
	Hypotension
	Hypothermia
	Chronic:
	Weight loss
	Failure to thrive
	Diarrhea
	Lethargy
	Abdominal distention
	Bloody diarrhea
	Dehydration
Laboratory/ imaging findings	Anemia
	Thrombocytosis
	Hypoalbuminemia
	Elevated white blood cell count with a left shift
	Eosinophilia
	Transient methemoglobinemia
Triggers	Milk/soy (most common)
	Solid food: rice, oats, barley, chicken, turkey, egg white, green peas, peanuts, sweet potatoes, white potatoes, fruits, fish, mollusks
Management	Elimination of food trigger
	Oral food challenge to evaluate for resolution

Table 26.7 Diagnostic criteria for patients presenting with possible FPIES

Acute FPIES	
Major criterion: Vomiting in the 1- to 4-h period after ingestion of the suspect food and absence of classic IgE-mediated allergic skin or respiratory symptoms	Minor criteria: 1. A second (or more) episode of repetitive vomiting after eating the same suspect food 2. Repetitive vomiting episode 1–4 h after eating a different food 3. Extreme lethargy with any suspected reaction 4. Marked pallor with any suspected reaction 5. Need for emergency department visit with any suspected reaction 6. Need for intravenous fluid support with any suspected reaction 7. Diarrhea in 24 h (usually 5–10 h) 8. Hypotension 9. Hypothermia
The diagnosis of FPIES requires that a patient meets the major criterion and ≥3 minor criteria. If only a single episode has occurred, a diagnostic OFC should be strongly considered to confirm the diagnosis, especially because viral gastroenteritis is so common in this age group. Furthermore, although not a criteria for diagnosis, it is important to recognize that acute FPIES reactions will typically completely resolve over a matter of hours compared with the usual several-day time course of gastroenteritis. The patient should be asymptomatic and growing normally when the offending food is eliminated from the diet.	
Chronic FPIES	
Severe presentation: When the offending food is ingested on a regular basis (e.g., infant formula); intermittent but progressive vomiting and diarrhea (occasionally with blood) develop, sometimes with dehydration and metabolic acidosis. Milder presentation: Lower doses of the problem food (e.g., solid foods or food allergens in breast milk) lead to intermittent vomiting and/or diarrhea, usually with poor weight gain/FTT but without dehydration or metabolic acidosis.	The most important criterion for chronic FPIES diagnosis is resolution of the symptoms within days after elimination of the offending food(s) and acute recurrence of symptoms when the food is reintroduced, onset of vomiting in 1–4 h, diarrhea in 24 h (usually 5–10 h). Without confirmatory challenge, the diagnosis of chronic FPIES remains presumptive.

Reproduced from Nowak-Węgrzyn et al. [54]

ative food, infants present with profuse, repetitive vomiting and may experience dehydration and lethargy. Diarrhea may occur several hours following the vomiting. In the chronic form, with continued ingestion of the allergen, infants develop weight loss and failure to thrive. Cow's milk and soy are the most common triggers in formula-fed infants. Symptoms begin 1–4 weeks after introduction of cow's milk or soy. Solid food FPIES is less common than milk/soy FPIES, and presents later, usually at 4–7 months of age, most often when weaning a breast-fed infant. Solid food FPIES triggers include most often rice and oat, but many foods have been implicated including barley, chicken, turkey, egg white, green peas, peanuts, sweet potatoes, white potatoes, fruits, fish, and mollusks. Of those infants diagnosed with solid food FPIES, 65% carried a prior diagnosis of milk/soy FPIES and 80% reacted to more than one food. Overall, 75% of infants presenting with FPIES appear acutely ill, and 15% become hypotensive, requiring hospitalization. Laboratory findings may include anemia, thrombocytosis, hypoalbuminemia, and an elevated white blood cell count with a left shift and eosinophilia. Transient methemoglobinemia and intramural gas have also been reported [54]. Table 26.6 shows key features of FPIES and Table 26.7 describes diagnostic criteria for acute and chronic FPIES. While the exact pathophysiologic mechanism for FPIES has yet to be elucidated, it is thought to be a cell-mediated disorder. Studies have shown increased levels of tumor necrosis factor-α, increased numbers of circulating blood mononuclear cells, and decreased intestinal mucosal expression of transforming growth factor-β receptors in association with FPIES [54].

Natural Course

Resolution of FPIES appears to be population dependent, particularly for cow's milk and soy. A study by Caubet et al. conducted in the United States found that 20% of patients with cow's milk FPIES resolved by 3 years of age [55]. Interestingly, a Korean cohort showed more than 60% resolution by 10 months of age and an Israeli birth cohort showed 90% resolution by 30 months [56–58]. Caubet et al. also found that 20% of cases of soy FPIES resolved by 3 years of age, whereas in the Korean cohort more than 90% of children showed resolution of soy FPIES by 10 months of age [55, 57]. These differences are likely explained by the higher proportion of subjects with detectable food-specific IgE levels and atopic dermatitis among the subjects who were referred to a major allergy center and the differences in methodology compared with the Israeli and Korean studies. Caubet et al. found that 65.5% of children with FPIES to grains resolved by 5 years of age, whereas resolution of FPIES to meat and fish/shellfish took more time (50% and 0% at 5 years of age, respectively) [55].

Diagnosis

History, clinical presentation, and at times oral food challenges are useful in the diagnosis of FPIES. A positive oral food challenge is considered the gold standard of diagnosis. However, if infants demonstrate a fitting history and presentation, an oral food challenge can be avoided if the symptoms resolve after removal of the suspected causative food. While >90% of patients do not have detectable serum-specific IgE to foods at the time of diagnosis, those that do have such antibodies are more likely to experience a protracted course of FPIES or develop acute allergic reactions [56, 59, 60].

Differential Diagnosis

In its acute form, FPIES is frequently mistaken for viral gastroenteritis, sepsis, or food poisoning. The intramural gas sometimes seen on abdominal radiographs mimics that seen in necrotizing enterocolitis. Methemoglobinemia often raises concern for a metabolic disorder.

Management

In acute cases, aggressive intravenous fluid administration is the mainstay of management. Other therapies for acute symptoms may include steroids and ondansetron [56, 61]. Methemoglobinemia may be treated with methylene blue. Chronic symptoms usually improve within 3–10 days after eliminating cow's milk from the diet and beginning a casein hydrolysate-based formula [59]. See Table 26.8 for further management recommendations for mild, moderate, and severe cases of FPIES.

Food Protein Induced Enteropathy

Food-protein-induced enteropathy is a disorder described decades ago and was characterized by vomiting, diarrhea, malabsorption, failure to thrive, and anemia in an infant [52]. Cow's milk protein was the most likely causative agent, particularly in infants fed intact cow's milk prior to 9 months of age. This syndrome has also been described in infants following an episode of gastroenteritis and in response to other foods, including eggs, rice, fish, shellfish, and poultry [53, 62]. Endoscopy with biopsy was required for diagnosis. Small bowel biopsies reveal patchy villous atrophy with a cellular infiltrate [63]. Affected infants would abstain from the causative food to bring about symptom resolution. Spontaneous resolution occurs typically by 2 years of age. This presentation has not been noted in the literature for decades. The diagnosis may have been subsumed by newer categorization of chronic FPIES or eosinophilic gastrointestinal disease.

Food Protein Induced Allergic Proctocolitis

Food-protein-induced allergic proctocolitis is characterized by blood and mucus in the stool of an otherwise healthy infant. Infants may be fussy or have increased frequency of bowel movements. The causative agent is usually milk in the maternal diet of a breast-fed infant, although egg, soy, and corn have been implicated as well [64]. The diagnosis is clinical, but rectal biopsy can be performed to confirm eosinophilic inflammation. The bleeding does not typically result in anemia. Maternal dietary elimination of the causal agent leads to resolution of symptoms within 72 h. The problem generally resolves by 1 year of age. The benign nature of the symptoms can allow re-trial of the potential trigger food protein weeks or a few months after resolution. The primary alternative explanation for these symptoms is infection. This diagnosis may be quite commonly made by primary care physicians and overdiagnosis is a concern because of benign causes of mild rectal bleeding in infants [65]. Inappropriate prolonged dietary elimination might be associated with onset of typical IgE-mediated food allergy [66].

Table 26.8 Management of acute FPIES episode at the medical facility

Presenting Symptoms		
Mild	Moderate	Severe
Symptoms		
1–2 Episodes of emesis No lethargy	>3 Episodes of emesis and mild lethargy	>3 Episodes of emesis, with severe lethargy, hypotonia, ashen or cyanotic appearance
Management		
1. Attempt oral rehydration (e.g., breastfeeding or clear fluids) 2. If age 6 mo and older: consider ondansetron intramuscular, 0.15 mg/kg/dose; maximum, 16 mg/dose 3. Monitor for resolution about 4–6 h from the onset of a reaction	1. If age greater than 6 mo: administer ondansetron intramuscular 0.15 mg/kg/dose; maximum, 16 mg/dose 2. Consider placing a peripheral intravenous line for normal saline bolus 20 mL/kg, repeat as needed 3. Transfer the patient to the emergency department or intensive care unit in case of persistent or severe hypotension, shock, extreme lethargy, or respiratory distress 4. Monitor vital signs 5. Monitor for resolution at least 4–6 h from the onset of a reaction 6. Discharge home if patient is able to tolerate clear liquids	1. Place a peripheral intravenous line and administer normal saline bolus, 20 mL/kg rapidly; repeat as needed to correct hypotension 2. If age 6 mo and older: administer intravenous ondansetron, 0.15 mg/kg/dose; maximum, 16 mg/dose 3. If placement of intravenous line is delayed because of difficult access and age is 6 mo or older, administer ondansetron intramuscular, 0.15 mg/kg/dose; maximum, 16 mg/dose 4. Consider administering intravenous methylprednisolone, 1 mg/kg; maximum, 60–80 mg/dose 5. Monitor and correct acid base and electrolyte abnormalities 6. Correct methemoglobinemia, if present 7. Monitor vital signs 8. Discharge after 4–6 h from the onset of a reaction when the patient is back to baseline and is tolerating oral fluids 9. Transfer the patient to the emergency department or intensive care unit for further management in case of persistent or severe hypotension, shock, extreme lethargy, respiratory distress
Strong consideration should be lent to performing food challenges in children with a history of severe FPIES in the hospital or other monitored setting with immediate availability of intravenous resuscitation. Oral challenges in the physician's office can be considered in patients with no history of a severe FPIES reaction, although caution should be urged because there are no data that can predict the future severity of FPIES reactions.		

Reproduced from Nowak-Węgrzyn et al. [54]

Mixed IgE- and Cell-Mediated Disorders

Atopic dermatitis (also referred to as eczema), eosinophilic esophagitis, and eosinophilic gastroenteritis are disorders that have both IgE- and cell-mediated components. In up to 40% of patients with atopic dermatitis, food allergy may lead to increased erythema and pruritus of eczematous lesions [23, 25, 26]. IgE-mediated flares occur within minutes to a few hours, while cell-mediated reactions may take up to several days to manifest themselves [67, 68]. Elimination of the suspected food allergen leads to improvement.

Eosinophilic gastrointestinal disorders are described in detail in other chapters. Briefly, eosinophilic infiltration of the gastrointestinal tract may result in dysphagia, vomiting, abdominal pain, poor growth, and food impaction and are the hallmarks of the eosinophilic gastrointestinal disorders (eosinophilic esophagitis and eosinophilic gastroenteropathy). A significant portion of patients with these disorders have other allergic diseases, and food is a primary trigger. In the case of eosinophilic esophagitis, elimination of foods to which the child has demonstrated sensitivity can result in both clinical and histological improvement. Similarly, elimination diets may show benefit in eosinophilic gastroenteropathy. The role of allergy testing remains controversial, but skin tests (including atopy patch tests) and serum tests may be helpful in guiding elimination diets and the means to reintroduce foods that were excluded from the diet [69, 70].

Diagnostic Evaluation of Food Allergy

Diagnostic evaluation of food allergy includes the history and physical examination, skin prick testing, serum-specific IgE testing, food elimination diets, and oral food challenges. The clinical history is paramount in diagnosing food allergy, as the pretest probability of food allergy determines what further testing is necessary. The history also discloses whether the illness is likely IgE antibody-mediated or not.

History and Physical Examination

The medical history plays a central role in determining which further steps in evaluation need to be performed. The symptoms and their temporal relation to the ingestion of food are particularly important. Acute IgE-mediated food-allergic reactions generally occur within seconds to minutes of ingestion of the food allergen. It is quite uncommon for these reactions to begin more than 2 h after the ingestion of the food. Chronic or delayed reactions may be cell-mediated and simple tests may not help to identify triggers—elimination diets and oral food challenges may be required.

With regard to IgE-mediated reactions, the time to resolution of symptoms should be noted. Particularly in the case of new-onset urticaria, in which 80% of cases are due to causes other than food allergy, [71] hives lingering longer than 24–48 h are unlikely to result from food allergy, unless the suspected allergen has been repeatedly ingested concurrent with the urticaria. Hives lasting more than a day or two should raise suspicion for a viral or other process rather than food allergy.

A food that has never been eaten before or is ingested rarely is much more likely to cause a reaction than foods that have been previously tolerated on a regular basis. Parents or other caregivers may have reached early closure regarding which substance was the causative food. The clinician must attempt to reconstruct an accurate history of all the food and drink ingested within 2 h prior to the reaction as best as possible. Dressings, beverages, side dishes, snacks, and sauces should be included in this evaluation. In addition, a careful review should take into consideration the possible cross-contact with a potential allergen. Cross-contact can be a cause of reactions at restaurants and buffet-style meals. A new allergy to a previously tolerated food is less likely than having a reaction to an ingredient that is not routinely ingested, or having had accidental exposure to a previously diagnosed allergen that was accidentally included in the meal that triggered a reaction.

If the patient has experienced allergic symptoms in the past, it is important to ask whether they had been consistently associated with the same food. Acute IgE-mediated allergic reactions generally occur every time the same quantity and preparation of an allergen is ingested. While trace amounts of protein can result in severe allergic reactions in particularly susceptible individuals, others have a threshold amount of protein that must be ingested before symptoms develop [72–75]. This threshold level can be as high as 10 g of the allergenic protein. In addition, cooking of foods induces conformational changes in certain proteins. For example, patients may react to less-heated forms of a food, such as the egg white in scrambled egg or French toast, but may not react to extensively heated forms of the same food (e.g., eggs baked in breads).

Depending on the patient's age, certain foods are more likely to be causative agents of an acute food-allergic reaction. In young children and infants, the following foods constitute 90% of IgE-mediated allergies: cow's milk, egg, soy, peanut, tree nuts, wheat, fish, shellfish, and sesame [1, 2]. In adolescents and adults, peanuts, tree nuts, fish, and shellfish are more common causes of serious acute reactions [15, 76, 77].

Besides the symptoms listed above that characterize acute reactions, the clinician should also note signs of chronic allergic processes, such as sinus venous congestion (and associated "allergic shiners"), horizontal nasal creases, boggy and pale nasal mucosa, or eczematous skin patches. While these signs of other allergic processes are not indicative of a food allergy in themselves, patients with other forms of atopy are more likely to experience food allergy, and therefore the presence of such signs increases the pretest probability of food allergy.

Tests for Food-Specific IgE

When the history and physical examination raise concern for possible IgE-mediated food allergy, skin prick testing and serum food-specific IgE testing can be helpful in investigating the potential allergens in question. Of paramount importance prior to selecting and interpreting these tests is the accurate medical history to determine pretest probability for IgE-mediated food allergy. A positive test (sensitization) to tolerated food(s) is common; therefore, a positive test cannot be solely used to diagnose food allergy. In addition, occasionally a test is negative despite true allergy. Therefore, negative tests with a compelling history should not be considered sufficient evidence of no allergy [1, 78, 79].

Skin Prick Testing

Skin prick testing is typically performed by allergist–immunologists. The allergen is introduced by scratching the surface of the skin and observing for a wheal and flare response, which is measured. Intradermal tests are not indicated as they are too sensitive and may induce systemic allergic reactions. Larger wheal size correlates with a greater concentration of food-specific IgE and greater likelihood of clinical allergy [80–82]. The size of the wheal does not correlate with severity of reaction. The sensitivity of skin prick testing is about 90%, the specificity is approximately 50% [83]. The skin of infants tends to be less sensitive than that of older children [84]. Given the high sensitivity of skin prick testing, it is a useful test for ruling out individual allergens in patients with a low pretest probability for food allergy to those specific allergens. However, performing skin prick testing to broad arrays of foods without attention to the medical history is not recommended, as the false-positive rate is high.

In Vitro Testing

In vitro testing methods, in contrast to skin tests, are not affected by antihistamine use, are not limited by skin conditions (such as urticaria, dermatographism, or eczema), and do not pose a risk of anaphylaxis. The first in vitro assays, termed "radioallergosorbent tests" (RAST), used radioactive isotopes to characterize relative IgE levels in a patient's serum. These have been replaced by fluorescent enzyme immunoassay (FEIA) tests, which determine serum-specific IgE levels to a variety of foods. Multiple in vitro assays have been developed, but results are not comparable across the various assays [85]. Most studies establishing normative serum-specific IgE cutoff values in children have used the Phadia ImmunoCAP FEIA (CAP-FEIA) system. Using this system, a prospective trial determined 96–100% positive predictive values for reaction during a food challenge for egg (≥7 kU_A/L), milk (≥15 kU_A/L), peanut (≥14 kU_A/L), and fish (≥20 kU_A/L) among 5-year-olds, with reactive thresholds being lower for children less than 2 years of age [86]. A study by Sindher et al. further analyzed biomarker thresholds for other allergens including almond (≥12.2 kU_A/L), hazelnut (≥14.6 kU_A/L), sesame (≥7.5 kU_A/L), walnut (≥ 13.5 kU_A/L), and wheat (≥43.1 kU_A/L) [87]. It is important to note that while skin prick testing and in vitro testing can determine the *likelihood* of a systemic reaction to ingestion of a particular food; they are unable to accurately predict the *severity* of that reaction should it occur. Newer generation tests evaluate specific IgE to specific proteins within a food. Foods are comprised of multiple proteins, of which those that resist degradation from heat or digestion are more likely to cause significant allergic reactions. Taking peanuts as an example, the peanut protein Ara h 8 is a labile protein homologous to a protein in birch tree pollen. When compared with the stable peanut storage protein Ara h 2, Ara h 8 is less likely to cause a significant allergic reaction. Elevated serum IgE levels to whole peanut can represent an allergy to Ara h 8, Ara h 2, or other peanut proteins. In individuals with allergy to whole peanut, performing component testing to determine which specific peanut proteins play a role in their food allergy is important in determining which patients may be appropriate for food challenge testing [78, 88].

Diagnostic Food Elimination Diets

In the case of acute IgE-mediated food allergy, eliminating the causative allergens prevents further reactions—this type of elimination diet represents treatment of the underlying disorder. In the case of chronic food allergy in which no causative food is identified on testing, particularly in cell-mediated and mixed IgE- and cell-mediated food allergy, elimination diets are pursued for both diagnostic and treatment purposes. There are three basic types of diagnostic elimination diets: focused elimination diets, oligoantigenic diets, and elemental formula diets.

In a focused elimination diet, based on clinical history, one or several suspect foods are removed from the child's diet. The child is then monitored for the improvement of symptoms. If the symptoms fail to improve over 2 weeks (in the case of chronic IgE-mediated processes) or over several weeks (in the case of cell-mediated and mixed IgE- and cell-mediated food allergies), the food(s) removed from the diet are unlikely to be the underlying cause of the child's symptoms.

In the case of a child in whom no potential causative foods can be identified, an oligoantigenic diet may be pursued. In this diet, several of the most common causative foods are removed from the child's diet while maintaining a nutritionally complete diet with other foods. One example is a diet consisting of rice, lamb, asparagus, spinach, lettuce, sweet potato, cooked apple, olive oil, sugar, and salt [89].

In rare circumstances, children with multiple suspected food allergy triggers and continued significant disease burden are placed on an elemental diet. In this type of diet, the child's food intake is limited to an extensively hydrolyzed or amino-acid-based formula. Such diets can have significant adverse consequences and should only be pursued under the supervision of an allergist and nutritionist familiar with such diets.

If the child's symptoms abate following introduction of an oligoantigenic or elemental diet, other foods are added one by one into the diet with careful monitoring for the return of symptoms. In addition to the complexity of maintaining nutritional adequacy in a food elimination diet, reintroduction of foods after a long period of elimination poses a risk of increased severity of reaction to foods to which specific IgE is present or develops [90]. Consequently, care should be taken to avoid unnecessary elimination of foods.

Food Challenges

A controlled oral food challenge is the gold standard for the diagnosis of food allergy. A food challenge is a physician-supervised ingestion of a single serving of potential allergen performed to confirm or refute the diagnosis of food allergy to that food. This test is generally used when history and supporting tests fail to confirm or refute an allergy. The test may be used for diagnosis of food allergy from any pathophysiology, IgE mediated or not.

A food challenge is performed for diagnostic purposes in the following scenarios:

- When several foods are being avoided, a food challenge can help determine foods that may be added back into the diet, particularly in the case of foods being avoided based on allergy testing alone (rather than a history of reaction).
- When IgE testing is negative, but a particular food is being avoided based on clinical history alone.
- When a cell-mediated or mixed IgE- and cell-mediated process is present (i.e., FPIES), a food challenge may be the only means of determining if an allergy is present.

- In addition, if serum IgE and skin prick test results appear to indicate that a particular allergy has resolved, a negative (or nonreactive) food challenge confirms allergy resolution [91].

Food Challenge Format

While a double-blind placebo-controlled food challenge is the gold standard for determining food allergy, in practice open food challenges and single-blind challenges tend to be more commonly pursued, as they reduce the amount of time required in clinic and overall cost of evaluation. A single-blind challenge, in which the observer, but not the child, is aware of which substance is placebo and which is the allergen, is useful in cases where a strong anxiety component is present. In an open food challenge, the child consumes the food in question in progressively increasing portion sizes every 10–15 min until the cumulative total of food given equals approximately one standard serving of the food. The patient is then observed for a predetermined period of time for delayed reactions prior to discharge home. If a reaction occurs, the challenge is halted, and the child treated for symptoms [91]. The physician performing the test, typically an allergist–immunologist, must be prepared to treat anaphylaxis.

Unproven Tests That Are Not Recommended

Food-specific IgG/IgG4, lymphocyte activation tests, kinesiology, sublingual or intradermal provocation tests, cytotoxic tests, or vega testing are not supported by scientific validation and should not be performed as part of food allergy evaluation [1].

Prevention of Food Allergy

A small body of evidence gathered at the beginning of the food allergy epidemic led to the recommendation to delay introduction of highly allergenic foods in order to try to prevent the development of food allergy. In 2008, due to lack of evidence and a continued increase in prevalence of food allergy, these recommendations were modified to acknowledge that data was insufficient to determine how timing of introduction of food allergens influenced the development of food allergy [92]. Finally, the LEAP (Learning Early About Peanut Allergy) study published in 2015 found that the early introduction of peanuts significantly decreased the frequency of the development of peanut allergy among children at high risk [93]. High-risk children were defined as those with severe eczema, egg allergy, or both. In this study, introduction of peanut was between 4 months and 11 months. Following this landmark trial, a consensus group recommended introducing peanut-containing products into the diets of "high-risk" infants early on in life (between 4 and 11 months of age) in countries where peanut allergy is prevalent because delaying the introduction of peanut can be associated with an increased risk of peanut allergy [94]. More recent guidelines for prevention encourage early incorporation of allergens in the infant diet [92, 95].

Dietary Treatment of Food Allergies

Avoidance

Food avoidance education is the cornerstone of food allergy management. Once it has been determined that a particular food must be eliminated from the diet, the child's family and all caregivers must undertake what amounts to a complete readjustment of one of the most basic daily habits—that of partaking in meals and snacks. Food elimination entails a careful evaluation of food packaging labels, safety in food preparation both in the restaurant and at home, and working with everyone from restaurant chefs, other children's parents, bakers, camp counselors, school cafeteria workers, and anyone else involved in the preparation of food to ensure that the allergen is not incorporated into any food consumed by the food-allergic child. Medications, vaccines, and cosmetics can also include food allergens. In highly sensitive individuals, less than a milligram of peanut, milk, or egg can cause a reaction [72–75].

Maternal Diet and Allergies in Breastfeeding

Food proteins can be detected in breast milk, and several cases have been reported of children experiencing reactions ranging from chronic atopic dermatitis to anaphylaxis due to maternal transfer of allergens through breast milk [96–99]. However, a tolerizing effect has been hypothesized in at least one study [100]. For infants with a history of reacting to a protein in the breast milk, strict maternal avoidance of the allergen is recommended. Many children with food allergy will be able to continue consumption of breast milk without removal of the allergen from the maternal diet.

Nutritional Issues in Food Allergy

When foods are being avoided due to allergy or potential allergy, care should be taken to assure that the nutritional needs of the affected child are addressed initially and revisited on a regular basis, as lower caloric intake and increased macro/micronutrient deficiency are more common in children with food allergy when compared with their age-matched peers without food allergy [101, 102]. The greater the number of allergens being avoided, the

greater the chance of a nutritional deficiency developing: Thus, greater diligence must be exercised to tailor food choices to ensure a well-balanced diet. In certain circumstances, this cannot be attained by incorporation of regularly available foods alone, and commercially prepared formulas may be necessary beyond the first year of life, particularly in children with cow's milk and/or soy allergies. As children with food allergies are more likely to suffer from inadequate growth and poor nutrition than their peers, a consult with a dietitian well versed in food allergy elimination diets is necessary in most children with food allergies that significantly affect protein, fat, or carbohydrate intake [1, 101–104].

Recent Advances in Food Allergy Management

Immunotherapy is an emerging management approach to patients with IgE-mediated food allergy. An allergic individual is administered very small amounts of allergen (e.g., peanuts) below the threshold of reaction and the dose is incrementally increased over months. The goal of therapy is to raise the reaction threshold to protect the individual from accidental ingestion or make an individual "bite-safe." These approaches have not been shown to be curative.

Different routes of administration are being studied and developed, including oral (OIT), sublingual (SLIT), subcutaneous (SCIT), and epicutaneous (EPIT, or a skin patch) immunotherapy. Although most studies have focused on single antigen desensitization, multi-food oral immunotherapy, where multiple food items are combined into a single oral immunotherapy course, is also being investigated for its safety and efficacy [105]. Most of these approaches are still in their investigational stages [106–108]. Other approaches under investigation also include vaccines, microbiome modulating agents, and biologics, each used either in conjunction with immunotherapy or as a single treatment. Currently the only FDA-approved treatment for food allergy is a standardized OIT product for peanut allergy.

Summary

- A food allergy is "an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food" [1].
- The nine major food allergens (milk, egg, peanut, tree nuts, soy, wheat, sesame, fish, and crustacean shellfish) account for 90% of allergic reactions.
- Food allergies may be IgE-mediated, cell-mediated, or "mixed" adverse immune responses.
- IgE-mediated reactions are typically sudden in onset following exposure to a food allergen, whereas cell-mediated responses may result in chronic inflammation or delayed symptoms.
- Acute IgE-mediated reactions can have cutaneous, ocular, gastrointestinal, respiratory, and/or cardiovascular symptoms.
- Anaphylaxis is defined as a serious allergic reaction that is rapid in onset and may cause death.
- Cell-mediated food-allergic reactions include FPIES, food-protein-induced enteropathy, food protein-induced proctitis and proctocolitis, and food-induced pulmonary hemosiderosis.
- FPIES is a cell-mediated gastrointestinal food allergy that typically manifests in infancy and generally resolves by 3 years of age. In the acute form, 1–3 h after ingestion of the causative food, infants present with profuse, repetitive vomiting and may experience dehydration and lethargy. Aggressive intravenous fluid administration, steroids, and ondansetron may be used to treat acute cases. Elimination of the causative food leads to chronic symptom resolution within 3–10 days.
- Food-protein-induced enteropathy is characterized by vomiting, diarrhea, malabsorption, failure to thrive, and anemia in an infant. Cow's milk protein is the most likely causative agent, and symptoms usually spontaneously resolve by 2 years of age.
- Food-protein-induced allergic proctocolitis is characterized by blood and mucus in the stool of an otherwise healthy infant. Maternal dietary elimination of the causal agent (usually cow's milk) leads to resolution of symptoms within 72 h. The problem generally resolves by 1 year of age.
- Atopic dermatitis, eosinophilic esophagitis, and eosinophilic gastroenteritis are disorders that can have both IgE- and cell-mediated components. Elimination diets may be helpful in these disorders.
- Diagnostic evaluation of food allergy includes the history and physical examination, skin prick testing, serum-specific IgE testing, food elimination diets, and food challenges. The clinical history is paramount in diagnosing food allergy, as the pretest probability of food allergy determines what further testing is necessary. The history also discloses whether the illness is likely to be IgE antibody-mediated or not.
- Skin prick testing and in vitro testing can determine the likelihood of an IgE-mediated systemic reaction to ingestion of a particular food, but they are unable to accurately predict the severity of that reaction should it occur. Positive predictive values for reaction during a food challenge have been established for in vitro testing to certain foods within certain age groups using a particular testing system.
- Food elimination diets play a treatment role in acute IgE-mediated food allergy and fulfill both diagnostic and

treatment roles in cell-mediated and mixed IgE- and cell-mediated food allergy.

- A food challenge is a physician-supervised ingestion of a single serving of potential allergen performed to confirm or refute the diagnosis of food allergy to that food.
- Immunotherapy is an emerging management approach to patients with IgE-mediated food allergy.

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Eosinophilic Gastrointestinal Disorders Beyond Eosinophilic Esophagitis

27

Eleni Koutri and Alexandra Papadopoulou

Introduction

Eosinophilic gastrointestinal disorders (EGIDs) are chronic inflammatory disorders of the gastrointestinal (GI) tract characterized clinically by symptoms related to the dysfunction of the affected segment(s) of the GI tract and histologically, by dense eosinophilic infiltration, in the absence of an identifiable secondary cause [1]. EGIDs are classified according to the affected segment(s) of the GI tract to eosinophilic esophagitis (EoE), eosinophilic gastritis (EG), eosinophilic gastroenteritis, and eosinophilic colitis (EC) [1, 2]. It should be noted, however, that it is not uncommon that multiple parts of the GI tract are involved in the inflammatory process, either simultaneously or sequentially [3, 4]. Furthermore, EGIDs beyond EoE are subclassified according to the depth of the eosinophilic inflammation through the wall of the GI tract to mucosal, muscular, or subserosal disease.

Due to the absence of biological markers, the diagnosis of EGIDs is based on clinical symptoms and on histological findings of eosinophilic inflammation, after the exclusion of a secondary cause of inflammation or a systemic disorder, which may be a challenging issue given the absence of strict histological criteria for EGIDs diagnosis (beyond EoE).

Currently, the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), and invited experts in the field have been working jointly on consensus guidelines on diagnostic criteria of EGIDs beyond the esophagus.

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Epidemiology

EGIDs are rare diseases. An electronic survey conducted by Spergel et al. to 10,874 US pediatric and adult allergists, immunologists, and gastroenterologists including a total of 1836 responses (17%) revealed a prevalence of EGE or EC in 28/100,000 population based on patients assessed by gastroenterologists and 2/100,000 by allergists and immunologists [5]. Later, Jensen et al. [6] estimated that there are less than 50,000 total patients with EGIDs in the United States, with the prevalence of EG estimated about 6.3/100,000 population, of EGE 8.4/100,000 and of EC 3.3/100,000 persons, while in children, the prevalence was 4.4, 10.7, and 4.3/100,000, respectively. More recently, a population-based database, established by Mansoor et al. extracting information from electronic health records from 26 major healthcare systems in United States between 1999 and 2017, estimated a prevalence of EGE in 5.1 per 100,000 individuals and of EC in 2.1 per 100,000 individuals [7].

EGIDs are reported to be more prevalent in Caucasians compared to African-Americans and Asians [7]. With regard to sex, Mansoor et al. [7] reported an increased prevalence of all EGIDs in females, Jensen et al. [6] of only EG, but not of EGE or EC, while Mark et al., reported that EC had a higher incidence in males [8].

According to early reports, EGE can affect patients of any age, from infancy through the seventh decade, but typically presents with a peak age of onset in the third decade of life [9–11]. Newer studies [7] reported EGE be more prevalent in children and adolescents (under 18 years of age) than in adults, with the highest prevalence compared to the other EGIDs, among children below the age of 5 years [6]. With regard to EC, some studies report greater prevalence in adults (older than 18 years of age) [7] than in children and adolescents, while others showed no age or gender differences [6].

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Esophageal involvement is not uncommon occurring in 10.6%, 12%, and 10.9% of patients with EG, EGE, and EC, respectively [6]. Furthermore, Jensen et al. [6] reported a high frequency of atopic comorbidities in patients (particularly in children), occurring in 38.5%, 45.6%, and 41.8% of patients with EG, EGE, and EC, respectively [6].

Pathophysiology

The eosinophils are present in all segments of the GI tract with the exception of esophagus [12], playing an important role in the immune homeostasis, while their absence is suggested to cause dysregulation of the mucosal barrier [13, 14]. The contribution of eosinophils to host defense is primarily based on the release of cationic proteins from cytoplasmic granules and various cytokines [12, 15]. However, the infiltration of the GI mucosa by an excessive amount of eosinophils, especially in association with changes in architecture and/or the presence of eosinophils in the deeper layers of the GI tract, is almost always pathologic.

The eosinophilic infiltration of the GI mucosa may be enhanced by the exposure to a food or other antigen [16], possibly through a Th_2 immune response [17]. Parasitic infections and other immune responses are also, likely to induce eosinophilic inflammation of the GI tract throughout important mediators like interleukin (IL)-5 [12]. In EGE, the recruitment and activation of eosinophils in the GI tract wall have been attributed to cytokine interleukins (IL)-3, IL-5 and granulocyte macrophage colony-stimulating factor [18], while the eotaxin family of chemokines has been reported to play a central role in regulating the accumulation of eosinophils in the lamina propria of the stomach and the small intestine, in response to antigen stimulation [19, 20].

The existing evidence suggests that a hypersensitivity reaction is involved in the pathogenesis of the disease [9, 21, 22], which is supported by the presence of peripheral eosinophilia, the elevated serum immunoglobulin E (IgE) levels [21, 23–25], and the prompt response to steroids [26, 27].

A study conducted at Mayo clinic revealed that 20 to 40% of patients with EGE had a history of an allergic diseases such as asthma, rhinitis, food allergy, drug allergy, and eczema [9]. Although the role of food allergy in the pathogenesis of EGE has not been well defined, the reports on improvement with an elemental or elimination diet [3, 28–30] support an atopic component in some patients.

Recently, Sato et al. [31] performed transcriptome analysis studies in children undergoing upper GI endoscopy for clinical symptoms suggestive of EGID between 2011 and 2016. The histological diagnosis of EG was based on finding of gastric mucosa eosinophilia ≥30 eosinophils per high power field (eos/hpf). The authors reported 1999 differentially expressed genes between patients with EG and the controls, including significant upregulation of eotaxin-3 (C-C *chemokine* ligand 26). More recently, Shoda et al. [32] showed in blood-based platforms from children with EG, increased eotaxin-3, thymus and activation-regulated chemokine, IL-5, and thymic stromal lymphopoietin levels. Upregulated gene cadherin 26 (CDH26), which is expressed by gastric epithelial cells, seems to play an important role in the pathogenesis of EG, as shown by other transcriptome analysis studies [33]. CDH26 binds to a4 and aE integrins regulating the adhesion and activation of the leukocytes, while, in vitro, it has been shown to inhibit CD4+ T cells, suggesting an important role as downregulating factor of inflammation [33]. Furthermore, other than eosinophils cells, such as mast cells and FOXP3-positive lymphocytes, seem to play also an important role in the disease's pathogenesis as their counts in the gastric mucosa of patients with EG have been increased to be excessive compared to the controls [4].

The pathogenesis of EC is even less clear. Several studies have shown similarly increased eosinophilic infiltration of the colonic mucosa of patients with IBD [34–37], that was much greater compared to patients with allergic conditions [38], while children with ulcerative colitis showed in their colonic biopsies from the recto sigmoid segment elevated levels of eotaxin-1 [37]. Torrente [39] et al. reported that patients with EC, but with IBD, had higher density of CD3+ T cells, eotaxin-2+ intraepithelial lymphocytes, and IgE+ cells in the lamina propria. The copresence in the colonic biopsies of inflammatory cell populations indicating chronic inflammation process in the absence of sheets of eosinophils is indicative of IBD, whereas the finding of degranulating eosinophils and mast cells in combination with IgE and tryptase deposits in perineural locations raise the suspicion for EC [39]. However, in some cases, the differential diagnosis of EC from IBD is challenging.

Clinical Manifestations (Table 27.1)

The clinical presentation of EGIDs varies depending on the location of the arising inflammation in the GI tract and the specific layer of the GI tract (mucosal, muscular, serosal) that is involved. Gastrointestinal mucosa is most commonly affected by the eosinophilic inflammation, but muscular and/or serosal layers can also be involved with different symptomatology and diagnostic approach [1, 17, 23, 40].

Table 27.1 Clinical manifestations of eosinophilic gastrointestinal disorders beyond eosinophilic esophagitis depending on the depth of inflammation through the GI tract wall

	Clinical symptoms and signs
Mucosal involvement	EG: Nausea, vomiting, retrosternal or epigastric pain, dyspepsia, gastrointestinal bleeding (hematemesis and/or melena) EGE: Nausea, vomiting, abdominal pain, diarrhea, failure to thrive/weight loss, protein loss, gastrointestinal bleeding (hematemesis and/or melena) EC: Abdominal pain, tenesmus, diarrhea with mucus, and/or blood
Muscular involvement	EG: Outlet obstruction mimicking pyloric stenosis EGE/EC: obstructive symptoms, intussusception, perforation
Subserosal involvement	EGE/EC: abdominal distention, ascites

EG Eosinophilic Gastritis, *EGE* Eosinophilic Gastroenteritis, *EC* Eosinophilic Colitis

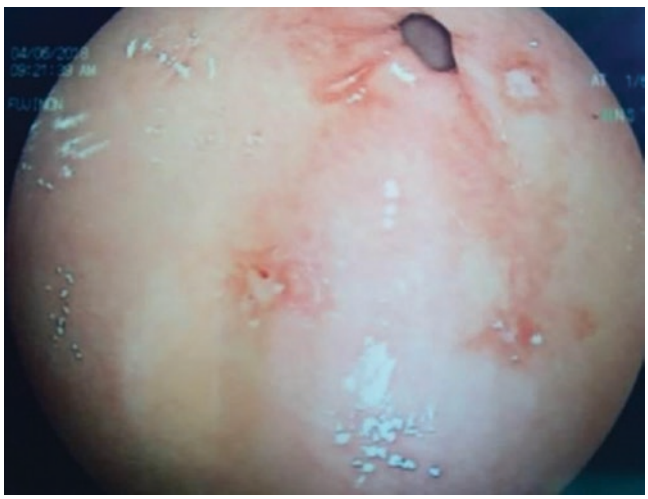

Fig. 27.1 Endoscopic features of eosinophilic gastritis [2]. Gastric antral erosions and ulcers in a child with eosinophilic gastroenteritis presented with epigastric pain and iron deficiency anemia. (From Koutri and Papadopoulou [2]. Adapted with permission from Kurger AG Basel)

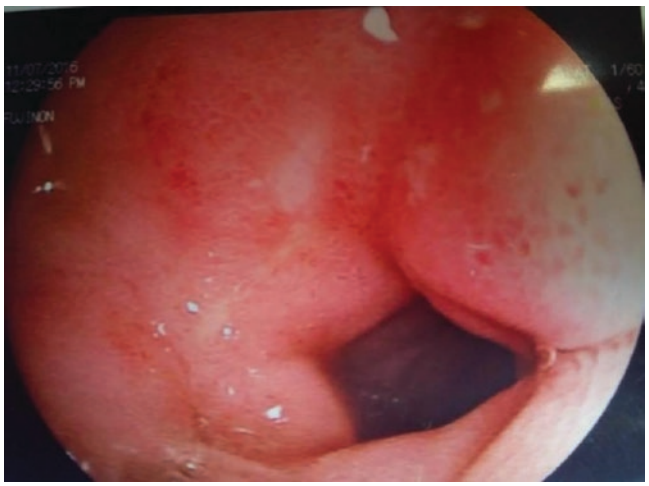

Fig. 27.2 Endoscopic features of eosinophilic gastroenteritis [2]. Duodenal bulb erosions in a child with eosinophilic gastroenteritis presented with hematemesis. (From Koutri and Papadopoulou [2]. Adapted with permission from Kurger AG Basel)

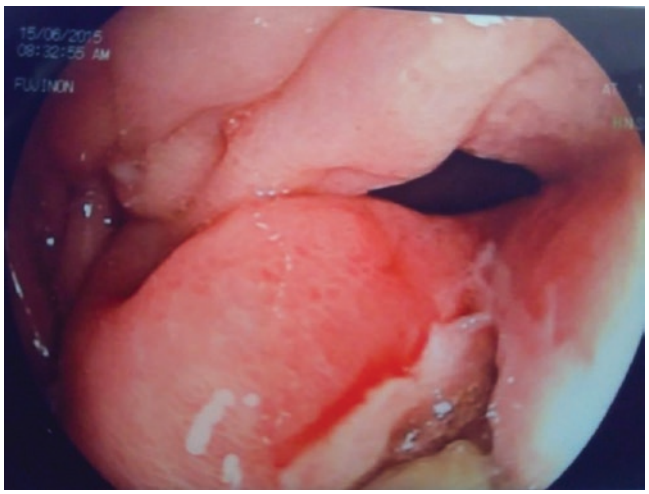

Fig. 27.3 Endoscopic features of eosinophilic gastroenteritis [2]. Duodenal giant ulcer in a child with eosinophilic gastroenteritis presenting with hematemesis. (From Koutri and Papadopoulou [2]. Adapted with permission from Kurger AG Basel)

Mucosal Disease (Figs. 27.1, 27.2, 27.3, 27.4, and 27.5) [2, 41, 42]

Mucosal EG presents with a variety of symptoms such as epigastric pain, nausea, vomiting, and early satiety [40], hematemesis and/or melena from gastric/duodenal erosions (Figs. 27.1 and 27.2) [2] or gastric/duodenal ulcers (Fig. 27.3) [2], while the laboratory findings include hypoalbuminemia, anemia, and peripheral blood eosinophilia [3, 4, 40, 43]. The presence of an isolated ulcer, not responding in proton pump inhibitor treatment, has also been described in adolescents [41, 44] (Fig. 27.4) [41] with possible perforation (Fig. 27.5) [42]. Jensen et al. reported as most common symptoms of EG in 774 patients, abdominal pain, chest pain/throat pain, and nausea/vomiting, while 10.2% of these patients had concomitant EoE, which possibly explained the presence of throat pain [6].

Mucosal EGE presents with nonspecific symptoms. In a retrospective study of 40 patients with mucosal subtype of EGE, the most common symptoms appeared to be abdominal pain, nausea, vomiting, early satiety, diarrhea, and occasionally bleeding and weight loss [9, 17, 26, 45, 46]. Patients with diffuse small intestine mucosal disease can develop malabsorption, malnutrition, anemia, protein-losing enteropathy, and failure to thrive [1, 23, 40, 47–49]. In a study of 44 patients with EGE, conducted by Reed et al., the most common symptoms were vomiting (71%) and abdominal

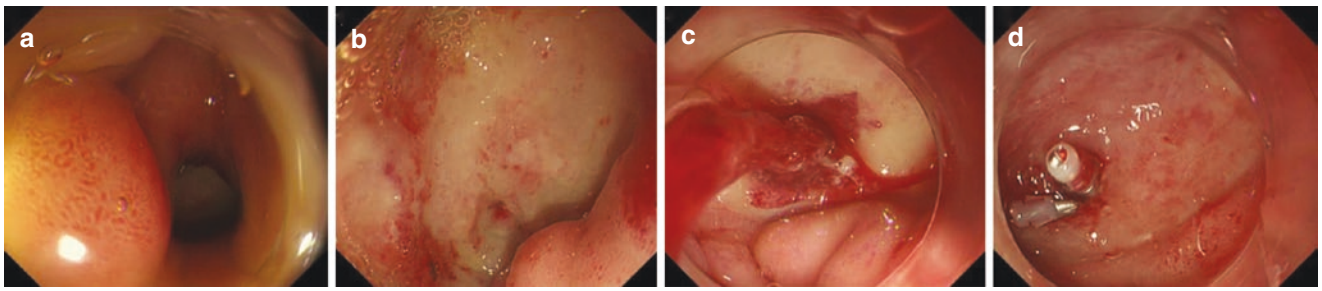

Fig. 27.4 (**a**–**d**) Hemorrhagic duodenal ulcer on an adolescent with eosinophilic gastroenteritis [41]. (**a**) Marked mucosal edema was observed in the duodenal bulb. An ulcer with a thickened, deep, white, moss-like appearance, and marked edema at its edge was present at the superior duodenal angulus. (**b**) Upper gastrointestinal endoscopic findings (approximately 1 week after the initial endoscopy). (**c**) Bleeding from an exposed vessel was noted. (**d**) Hemostasis was performed using a clip. (From Yamazaki et al. [41]. Reproduced with permission from Springer Nature)

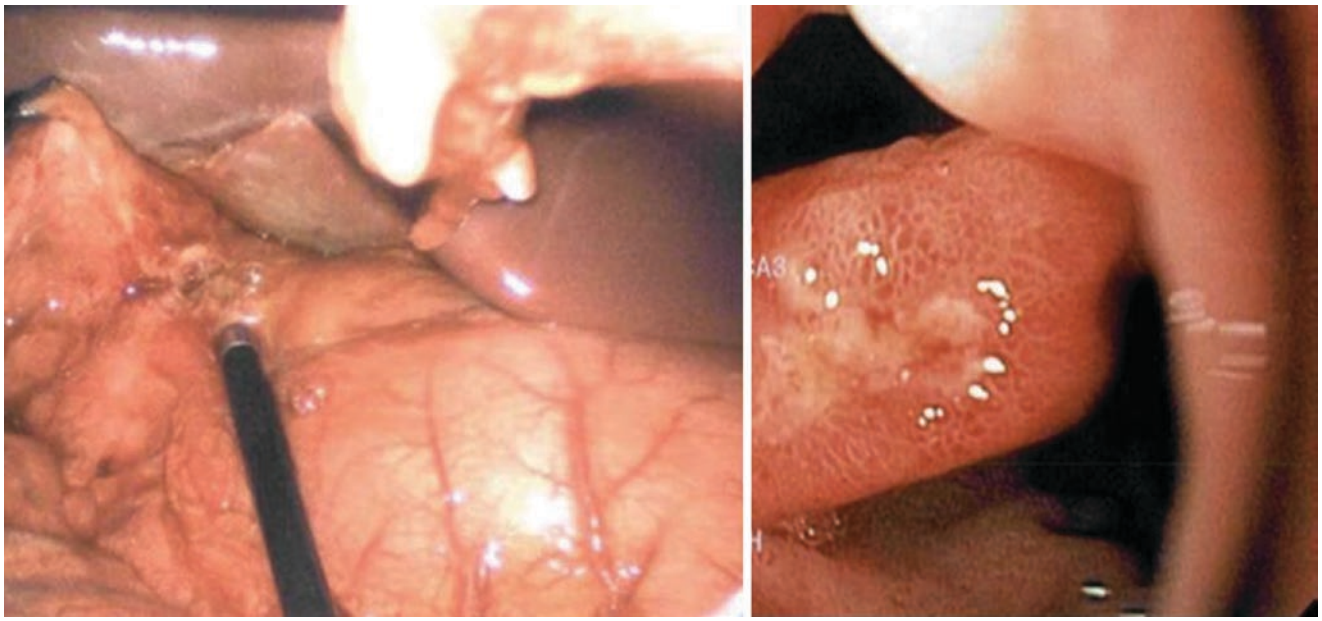

Fig. 27.5 (**a**–**b**) Perforated duodenal ulcer in a 16-year-old boy [42]. (**a**) Intraoperative photo depicting the perforated duodenal ulcer in the first portion of the duodenum. (**b**) Initial endoscopy showing residual duodenal ulceration after repair. (From Riggle et al. [42])

pain (62%) [48]. Jensen et al. reported abdominal pain, diarrhea, and nausea/vomiting as the most common symptoms among 954 patients with EGE [6]. Furthermore, it has been shown that approximately 80% of patients have symptoms for several years [50].

Mucosal EC manifests with abdominal pain, diarrhea, and even hematochezia although, in some circumstances, patients may be totally asymptomatic [6, 8, 34, 40].

Muscular Disease (Figs. 27.6, 27.7, 27.8, 27.9 and 27.10) [51–53]

The eosinophilic infiltration of the muscle layer of the GI tract may cause wall thickening and impaired motility with symptoms suggestive of obstruction such as nausea, vomiting, and abdominal distention [9, 23, 47, 52, 54, 55]. Eosinophilic infiltration of the muscular layer may cause

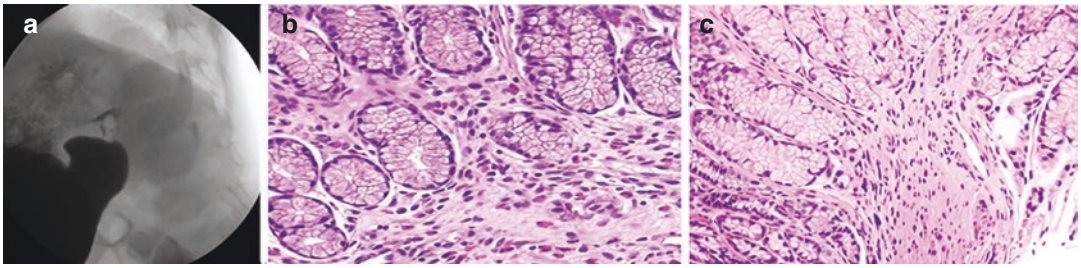

Fig. 27.6 Upper gastrointestinal follow through and gastric mucosal biopsy histology. (**a**) Minimal advancement of the contrast material through the pylorus (arrow). (**b–c**) Histologic images before and 5 d after steroid therapy. (**b**) Peripyloric antral sections showed prominent eosinophilic infiltration of the lamina propria (up to 30 eosinophils per single high-power field), with occasional degranulation (arrow) of eosinophilic content and infiltration of the muscularis mucosae. (**c**) Biopsies 5 d after intravenous steroid therapy demonstrated only a few eosinophils with a peak count of 2 eosinophils per high-power field (HE, ×40). (From Kellermayer et al. [52] Reproduced with permission by Baishideng Publishing Group Inc)

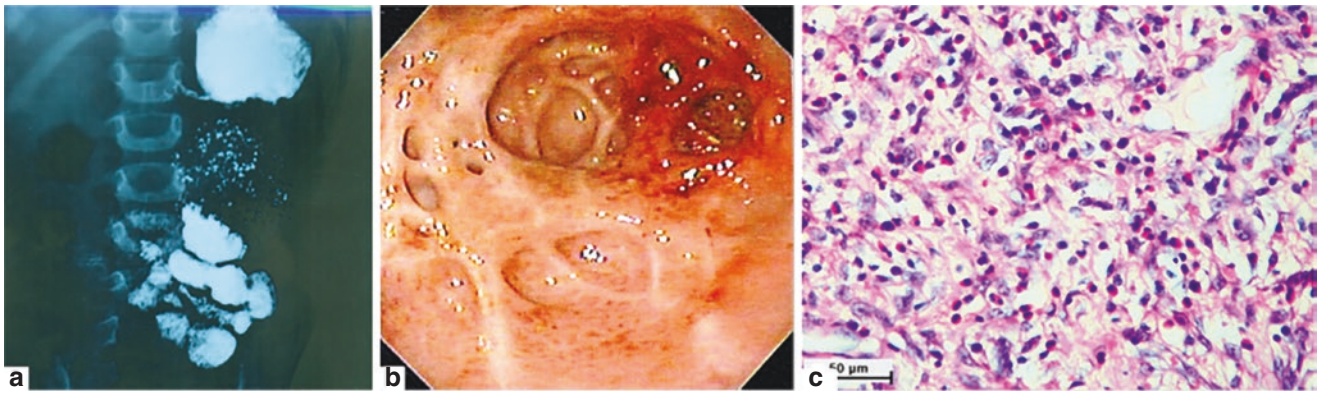

Fig. 27.7 (**a–c**) Gastric outlet obstruction on a 3-year-old girl with eosinophilic gastritis. (**a**) Barium meal X-ray showing absence of the duodenal bulb and the C-loop. Note that the distal stomach is also not visible. (**b**) Endoscopic visualization of the gastric mucosa showing formation of multiple diverticuli, erosion, and ulceration. (**c**) Dense infiltrates of eosinophils in muscle layer of the stomach (hematoxylin and eosin stain, ×400). (From Katiyar et al. [53] Reproduced with permission from Springer Nature)

dilatation of the bile ducts [51] or result in obstruction (and even perforation) of the gastric outlet, small bowel, and rarely the colon [9, 21–23, 25, 47, 56–58]. It should be noted, however, that in EGIDs beyond the esophagus, the presence of strictures is not that common as in EoE [44, 59].

Serosal/Subserosal Disease (Figs. 27.11 and 27.12) [60, 61]

In serosal/subserosal disease, patients may present with isolated ascites or ascites combined with symptoms of mucosal or muscular involvement [9, 60, 61]. An eosinophilic pleural effusion can also be present [47]. Features like cholangitis, pancreatitis [62], acute appendicitis, and eosinophilic splenitis have also been reported in literature.

Laboratory Findings

Peripheral blood eosinophilia occurs in 20–80% of patients [9] with eosinophil counts ranging from 5% to 35% with an average absolute eosinophil count of 1000 cells/μL [63]. Mucosal and serosal/subserosal EGE are usually associated with higher eosinophil counts compared to muscular EGE. EGE is often associated with iron deficiency anemia due to impaired iron absorption and/or occult gastrointestinal bleeding, especially in the mucosal subtype of the disease [23, 24, 47]. Hypoalbuminemia may occur due to the

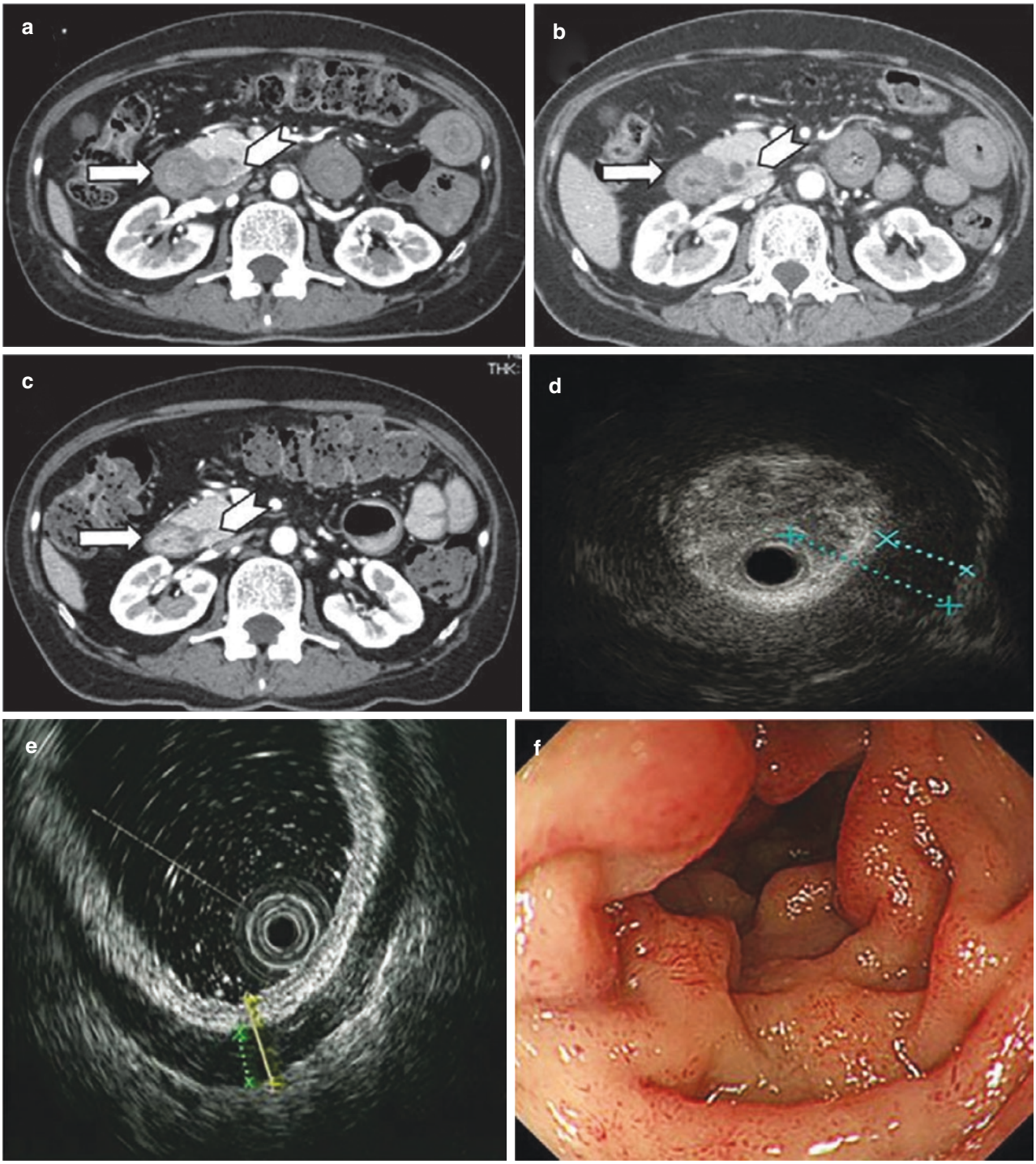

Fig. 27.8 (**a**–**h**) Contrast-enhanced computed tomography (CT) findings (**a**–**c**) and endoscopic ultrasound (EUS) findings of the duodenum (**d**–**e**). Upper gastrointestinal endoscopic examination findings (**f**–**h**). (**a**, **b**) CT revealed the thickening of the gastroduodenal mucosal wall (arrow). (**d**) EUS of the duodenum revealed extreme thickening of the mucosal and muscular walls. (**c**, **e**) Both findings were improved after steroid therapy. In contrast, (**b**) dilation of the bile duct (arrow head) and (**g**) narrowing of the lumen of the second part of duodenum diminished before steroid therapy. (**a**, **d**, **f**) On admission, (**b**, **g**) 5 days before steroid administration and (**c**, **e**, **h**) 2 weeks after steroid administration. (From Hamamoto et al. [51]. Reproduced with permission from The Japanese Society of Internal Medicine)

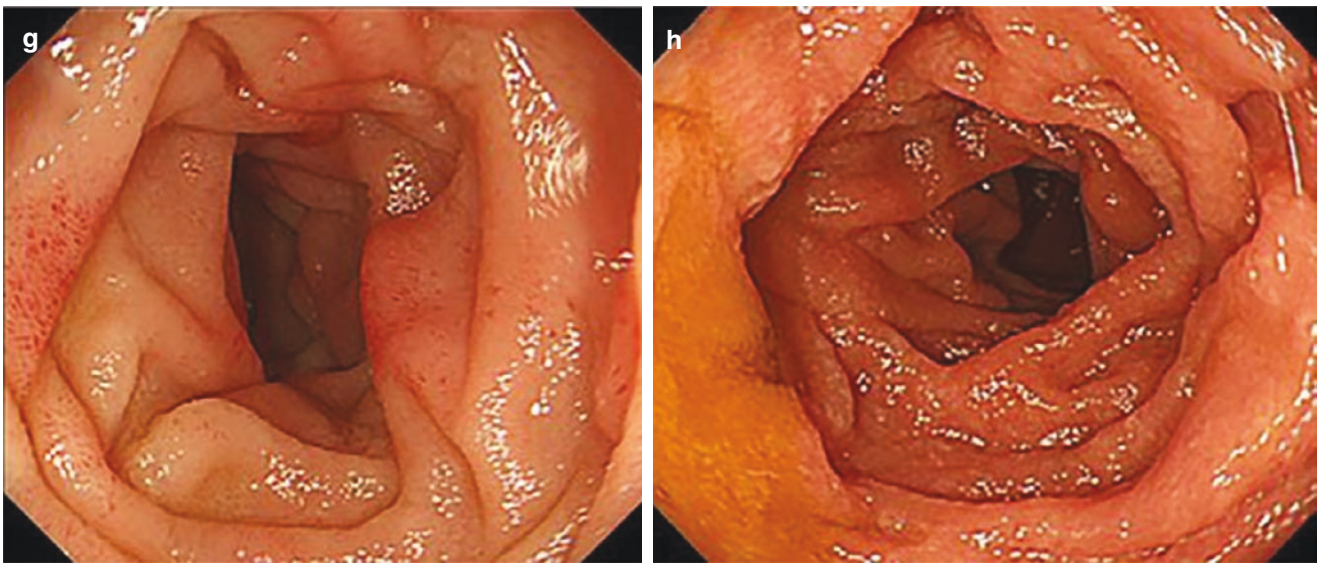

Fig. 27.8 (continued)

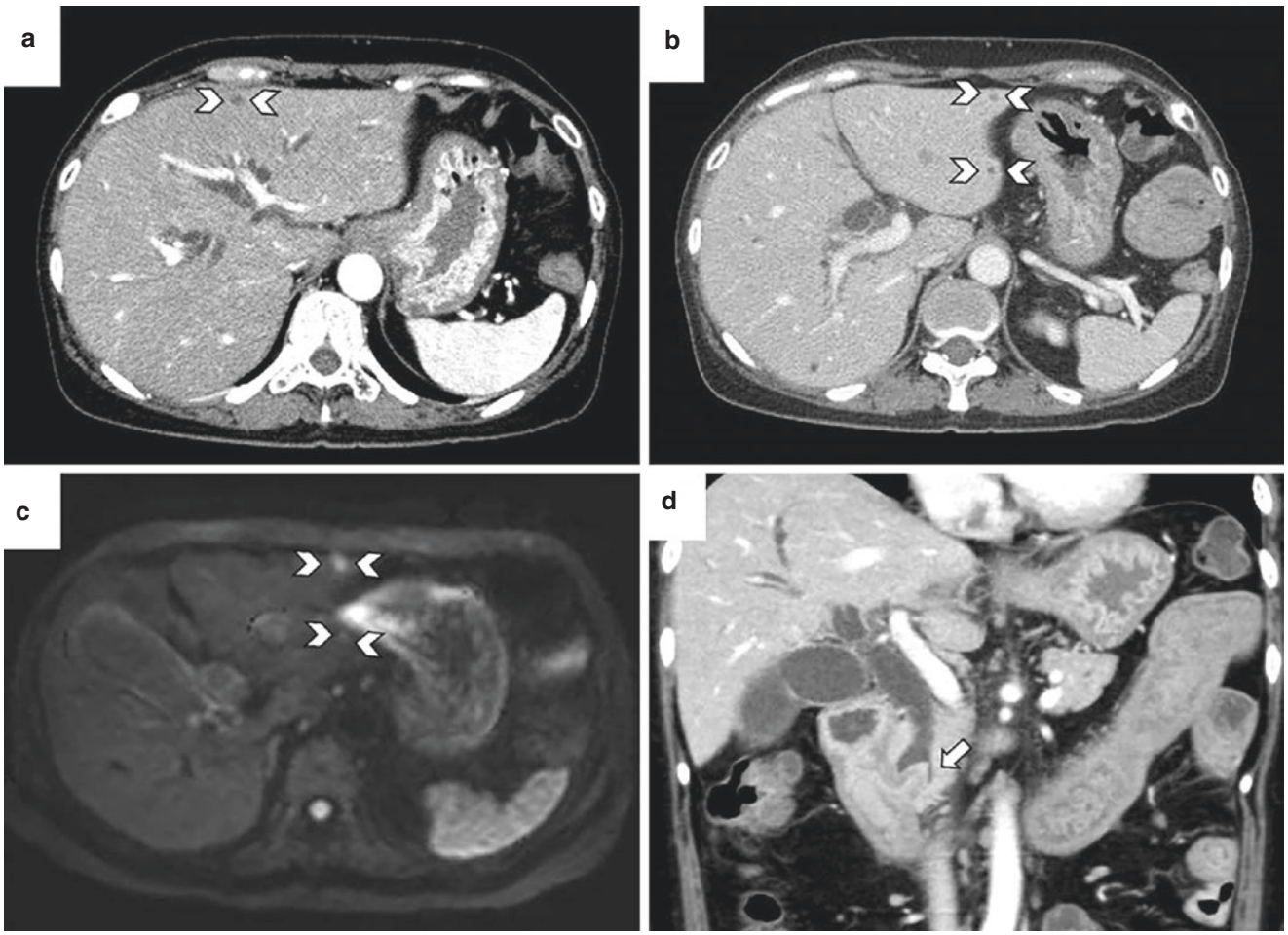

Fig. 27.9 (**a**–**e**) Abdominal contrast-enhanced computed tomography (CT) and dynamic contrast-enhanced magnetic resonance imaging (MRI) findings. (**a**, **b**) CT showed the common bile duct dilatation and microabscesses in the left lobe of the liver (arrowhead). (**c**) Regarding MRI, the abscesses showed a positive signal on diffusion-weighted imaging (arrowhead). (**d**) In the CT examination, invagination of the duodenal wall caused bile duct dilatation (arrow). (From Hamamoto et al. [51]. Reproduced with permission from The Japanese Society of Internal Medicine)

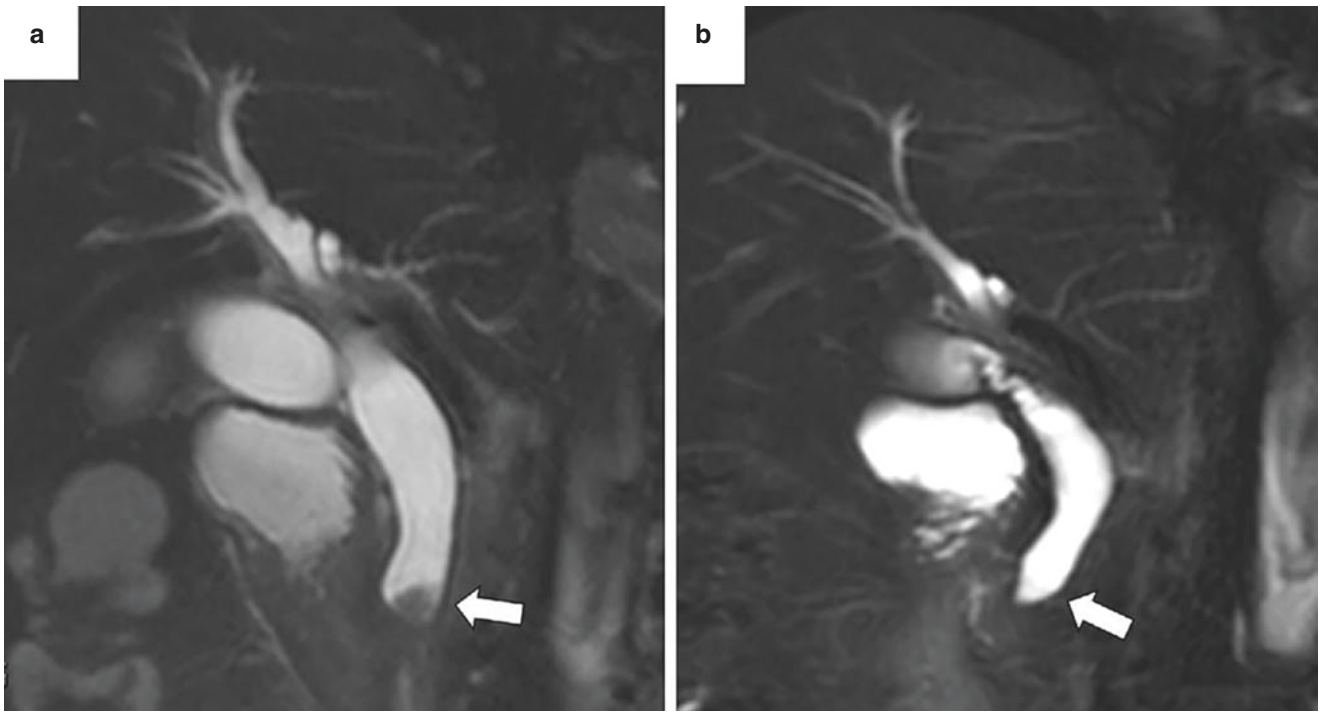

Fig. 27.10 Changes in the invagination of the duodenal wall during the clinical course. The changes were observed by magnetic resonance cholangiopancreatography. Before steroid therapy (**a**); after half a year of steroid therapy (**b**). (From Hamamoto et al. [51]. Reproduced with permission from The Japanese Society of Internal Medicine)

increased mucosal permeability and protein-losing enteropathy that can be assessed measuring fecal alpha 1-antitrypsin in a 24-h feces collection. Low levels of immunoglobulins can present consequently to the protein loss [9, 21, 23]. The erythrocyte sedimentation rate is usually normal or modesty elevated in approximately 25% of patients [9, 47].

Gastrointestinal Endoscopy (Figs. 27.1, 27.2, 27.3, 27.4, and 27.5) [2, 41, 42]

The endoscopy findings in EG or EGE may include nodular or polypoid appearance of the gastric mucosa, erythema, friability, and occasional ulcerative or erosive changes of either gastric (Fig. 27.1) [2] or duodenal mucosa (Fig. 27.2) [2], although not rare is the development of a giant ulcer that may cause severe abdominal bleeding and even perforation (Figs. 27.3, 27.4, and 27.5) [2, 41, 42], while in some circumstances, the mucosa may appear completely normal [2, 3, 4, 29, 43]. Newer reports in patients with EGE using wireless capsule endoscopy have shown salmon patch colored lesions with noticeable eosinophilic infiltration throughout their intestinal mucosa [65]. In patients with EC, the endoscopy findings include erythema, aphthous ulcers, erosions, whitish elevated lesions, or pale granular mucosa.

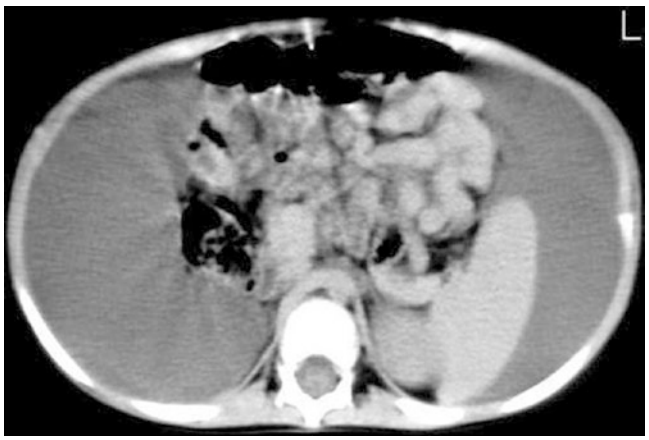

Fig. 27.11 CT scan findings on a 3-year-old boy with subserosal EGE [60]. CT scan shows a massive ascites displacing the intestine in the center of the abdomen in a 3-year-old patient with serosal subtype of EGE. (From Barabino et al. [60]. Reproduced with permission from Springer Nature)

It should be noted, however, that none of the above findings are specific for EGIDs.

In patients with subserosal involvement, abdominal ultrasound (US), abdominal computed axial tomography (CT), or magnetic resonance imaging (MRI) may reveal the presence of ascites (Figs. 27.11 and 27.12) [60, 61].

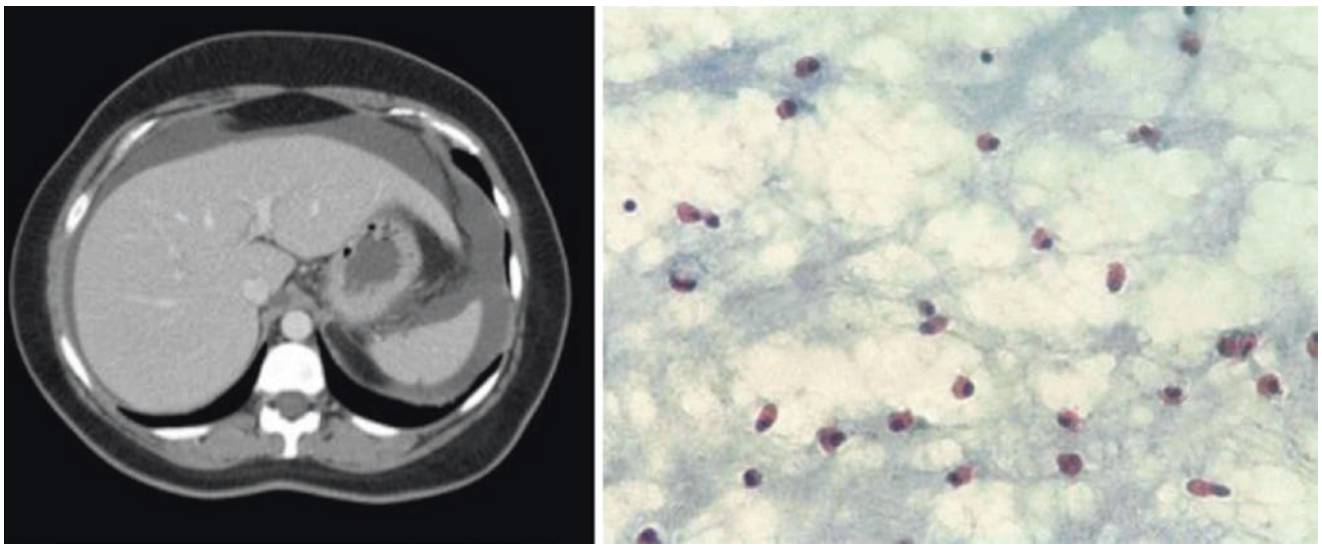

Fig. 27.12 (**a**–**b**) Abdominal computed axial tomography on an adult patient with subserosal EGE and histological examination of the ascitic fluid [61]. (**a**) Abdominal computed axial tomography showing ascites and gastric wall thickening. (**b**) Histological examination of ascitic fluid showing abundant cellularity with inflammatory characteristics with a predominance of eosinophils (40× magnification). (From Ferreira et al. [61]. Reproduced with permission from SMC media Srl)

Histology (Figs. 27.13, 27.14, 27.15, 27.16, 27.17, and 27.18) [2, 66]

In contrast to EoE, consensus diagnostic histological criteria for EGIDs beyond the esophagus are lacking [17, 40]. Various studies have defined differently pathological eosinophilic infiltration of different parts of the GI mucosa to justify the diagnosis of EGIDs in the clinical context [35, 43, 67, 68]. With regard to eosinophilic infiltration of the mucosa of different segments of the GI tract, the following counts were considered by several authors to justify the diagnosis of EGIDs in the clinical context: ≥30 eosinophils per high power field (eos/hpf) in ≥5 hpfs or ≥70eos/hpf in ≥3 hpfs in the gastric mucosa, for the diagnosis of EG [3, 43, 69, 70]; ≥52 eos/hpf in duodenal mucosa and ≥56 eos/hpf in ileum for the diagnosis of EGE [17, 59, 69, 70]; ≥100 eos/hpf in cecum and ascending colon, ≥84 eos/hpf in transverse and descending colon, and ≥64 eos/hpf in the mucosa of rectosigmoid area, for the diagnosis of EC [70]. Debrosse et al., however, assessed the amount and location of eosinophils in the GI tract of healthy children and reported much lower peak numbers of eosinophils in the "healthy" GI mucosa: ≤26 eos/hpf in the duodenum; ≤50 eos/hpf in the ascending colon; and ≤30 eos/hpf more distally [69].

It is not easy to explain the discrepancies in the eosinophilic counts in the GI mucosa reported by different authors. An important issue is the calculation of eosinophil counts per high power field the size of which depends on the technical parameters of the microscope such as the magnification of the objective lens and the diameter of the ocular. The diversity of the technical parameters of the commercially available microscopes may cause up to fivefold discrepancies in the eosinophil counts of the same biopsy sample. In our recent study [71] carried out in three major pediatric centers (Athens, Madrid, and Rome), histology slides (n = 1014) from GI biopsies taken from 155 children who had no organic disease (based on an extensive work up due to GI symptoms and the GI endoscopy) were reviewed and the eosinophilic counts were expressed per different sizes of hpfs (0.196 mm^2 and 0.306 mm^2) and as eos/mm^2 [71]. The study showed discrepancies in the peak counts of eosinophils in the same segments of the GI tract depending on the size of the hpf that was used for the calculation (Figs. 27.13 and 27.14) [71]. The above discrepancies highlight the importance of using eosinophil density (eos/m^2) instead of eos/hpf, that is a universally accepted tool, independent of the size of hpf [71]. Furthermore, in the above study, we found a significant geographical distribution in eosinophilic counts of the GI mucosa of children with no organic disease, but not differences among children with and those without the diagnosis of functional GI disorders based on Rome IV criteria [71].

Other histological abnormalities found in patients with EGIDs (Figs. 27.15 and 27.16) [2] may include the findings of eosinophilic sheets in expanded lamina propria, abundant intraepithelial eosinophils, eosinophil cryptitis/pititis or abscesses, or the presence of eosinophils in the muscularis mucosa and submucosa. In some circumstances, complete loss of intestinal villi (Fig. 27.17) [66], multiple layers involvement, submucosal edema, and fibrosis may develop consequently to diffuse intestinal inflammation [24, 66, 72].

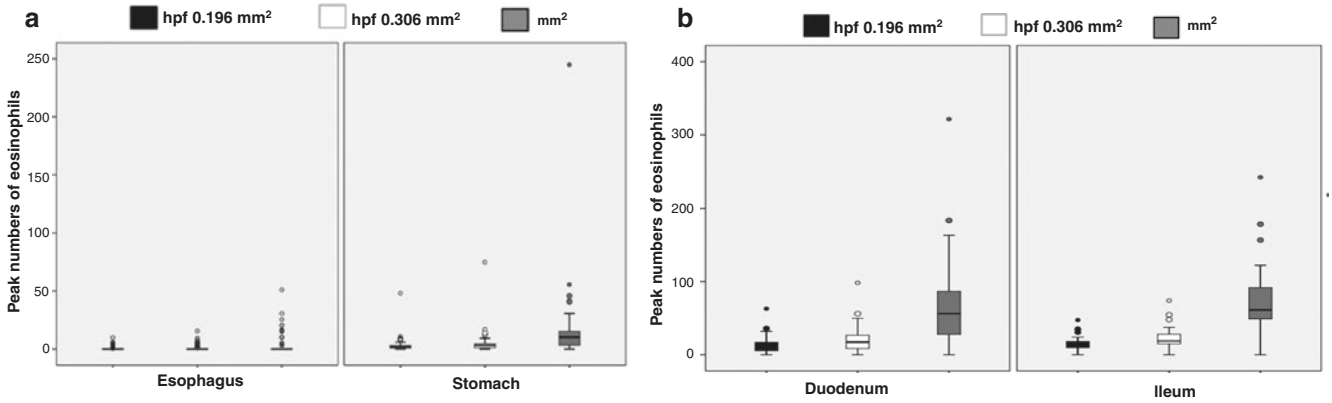

Fig. 27.13 (**a**, **b**) Peak numbers of eosinophils in the esophageal, gastric, duodenal, and ileal biopsies in children with no organic diseases [72]. (**a**) Median (IQR) of peak counts of eosinophils/high power field 0.196 mm^2 and 0.306 mm^2, and /mm^2 in the esophagus (111 biopsies) and the stomach (111 biopsies) was as follows: esophagus: 0 (0–0), 0 (0–0), and 0 (0–0), respectively; stomach: 2 (0.6–3.0), 3.1 (1.0–4.7), and 10.2 (3.3–15.3), respectively. (**b**) Median (IQR) of peak counts of eosinophils /high power field 0.196 mm^2 and 0.306 mm^2, and /mm^2 in the duodenum (111 biopsies) and ileum (44 biopsies) was as follows: duodenum: 11.0 (5.1–17.0), 17.2 (8.0–26.5), and 56.1 (26.1–86.7), respectively; ileum: 12.0 (9.6–18.0), 18.7 (15.0–28.1), and 61.2 (49.0–91.8), respectively (From Koutri et al. [71]. Reproduced with permission from Annals of Gastroenterology)

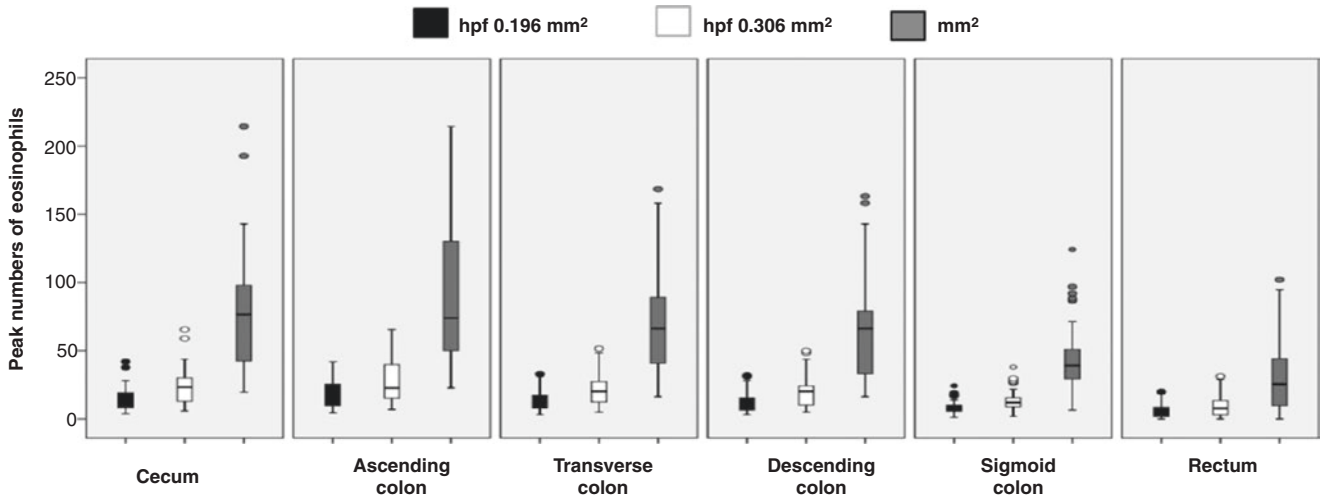

Fig. 27.14 Peak numbers of eosinophils in the colonic biopsies in the whole cohort of children. Median (IQR) of peak counts of eosinophils / high power field 0.196 mm^2 and 0.306 mm^2, and /mm^2 in the cecum (37 biopsies), ascending colon (28 biopsies), transverse colon (44 biopsies), descending colon (31 biopsies), sigmoid colon (37 biopsies), and rectum (41 biopsies) was as follows: cecum: 15.0 (8.0–19.5), 23.4 (12.5–30.5), and 76.5 (40.9–99.7), respectively; ascending colon: 14.5 (9.7–25.8), 22.8 (15.2–40.2), and 73.9 (49.5–131.4), respectively; transverse colon: 13.0 (8.0–17.9), 20.3 (12.5–28.0), and 66.3 (40.8–91.5), respectively; descending colon: 13.0 (6.0–16.0), 20.3 (9.4–24.9), and 66.3 (30.6–81.6), respectively; sigmoid colon: 7.6 (5.4–10.0), 12.0 (8.5–15.6), and 39.2 (27.8–51.0), respectively; rectum: 5.0 (1.9–8.9), 7.8 (3.0–14.0), and 25.5 (9.8–45.8), respectively (From Koutri et al. [71]. Reproduced with permission from Annals of Gastroenterology)

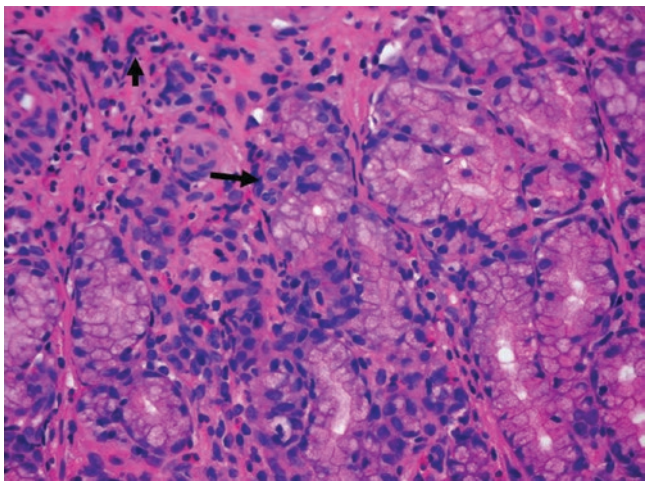

Fig. 27.15 Histological features of eosinophilic gastritis [2]. Aggregates of eosinophils near the muscularis mucosa [small arrow]. Eosinophilic infiltration of pyloric glands [long arrow]. (From Koutri and Papadopoulou [2]. Adapted with permission from Kurger AG Basel)

In patients with EC, the findings in colonic biopsies (Fig. 27.18) [2] may include apart from increased eosinophilic density, eosinophilic cryptitis/crypt abscesses, abnormalities in the architecture of the crypts, increased intraepithelial eosinophils, and the presence of eosinophils in the muscularis mucosa and submucosa [66].

In order not to miss the diagnosis, multiple biopsies should be taken from both normal- and abnormal-appearing mucosa since even a normal-appearing mucosa can be infiltrated by eosinophils and demonstrate inflammation [73].

In patients with muscular or subserosal type of EGE, mucosal biopsies can be normal [9, 73]. Thus, negative endoscopic mucosal biopsies do not definitively exclude muscular or subserosal disease. In that case, laparoscopic full-thickness biopsy should be performed to establish the diagnosis of EGID. In patients with intestinal wall thickening and/or obstruction, laparoscopic full-thickness biopsy is important to exclude a possible underlying malignancy.

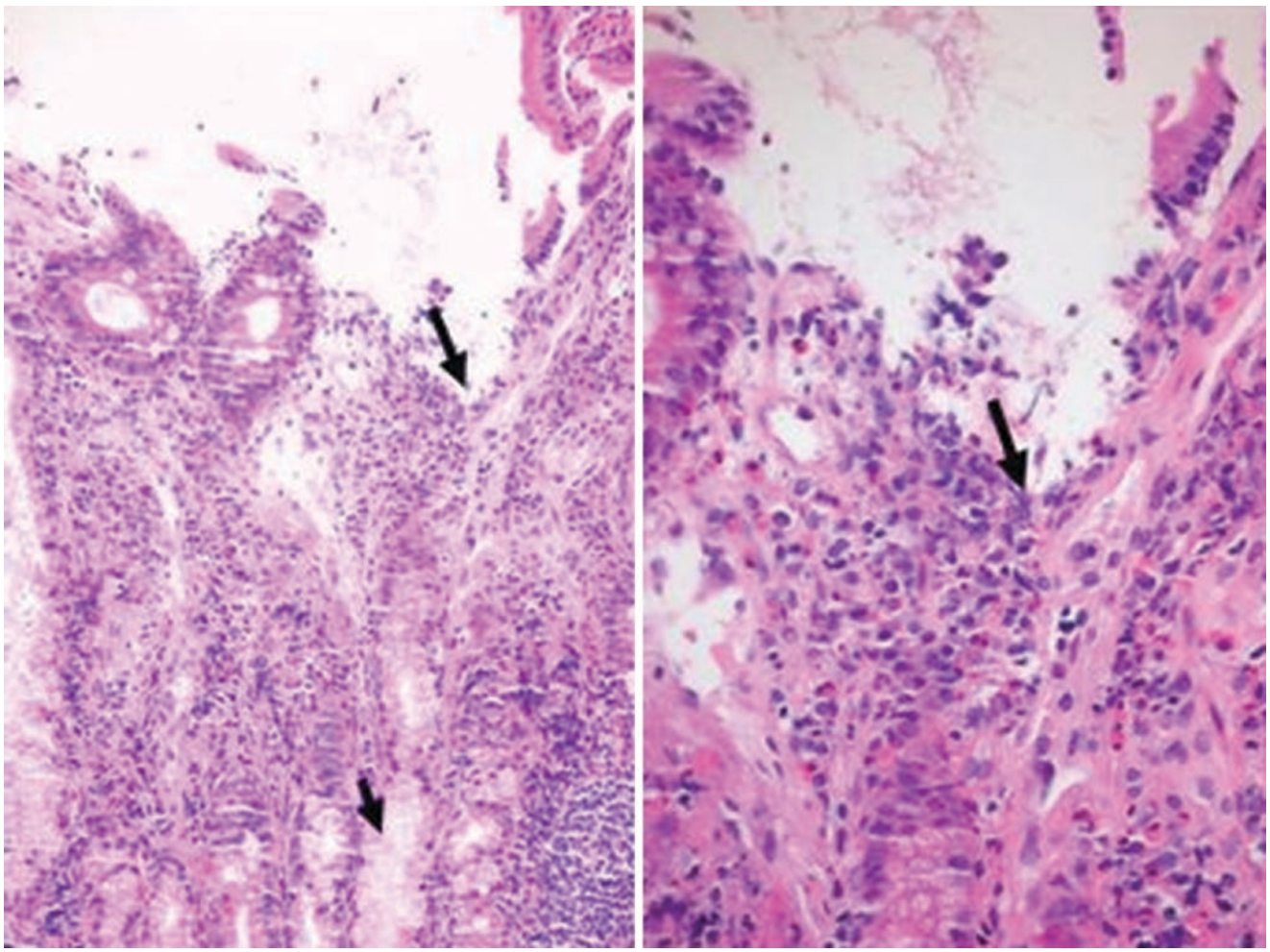

Fig. 27.16 (**a**, **b**) Histological features of eosinophilic gastroenteritis (duodenal biopsies) [2]. (**a**) Erosion and aggregates of eosinophils [long arrow]. Eosinophilic infiltration of the Brunner glands [small arrow]. (**b**) Aggregates of eosinophils at the deep part of the crypts with degranulation [long arrow]. Eosinophilic infiltration of the crypts [small arrow]. (From Koutri and Papadopoulou [2]. Adapted with permission from Kurger AG Basel)

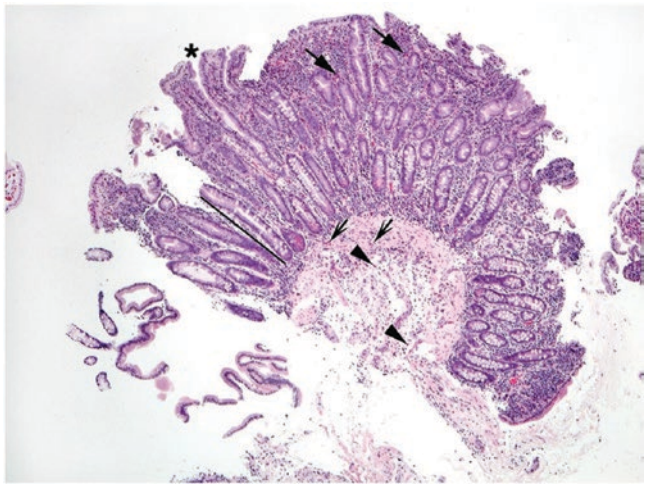

Fig. 27.17 Eosinophilic gastroenteritis—duodenal biopsy [66]. This duodenal biopsy shows few preserved short villi (asterisk), elongated crypts (bar), and numerous eosinophils in the lamina propria (arrows), muscularis mucosa (shaded arrows), and submucosa (arrowheads). (From Collins et al. [66])

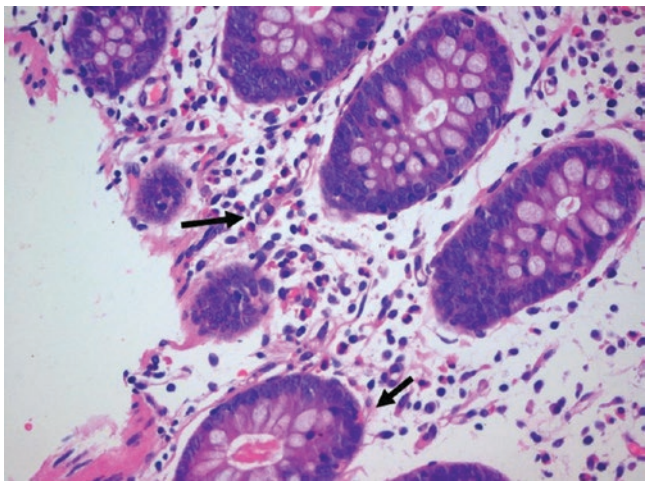

Fig. 27.18 Colonic biopsies histology on a child with eosinophilic colitis (histology) [2]. Aggregates of eosinophils at the deep part of the colonic crypts with degranulation [long arrow]. Eosinophilic infiltration of colonic crypts [small arrow]. (From Koutri and Papadopoulou [2]. Adapted with permission from Kurger AG Basel)

In conclusion, the location and depth of the obtained GI biopsies, the technical parameters of the microscope, and the analyses used for the examination of the histological samples, as well as the knowledge of the geographic variation in the normal amount of eosinophils in healthy GI tract, are all important factors to consider when interpreting the histological findings [1, 71]. Taking into account that there are no well-determined histological criteria for the diagnosis of EGIDs beyond EoE and until the consensus recommendations on diagnostic criteria of EGIDs by the ESPGHAN and NASPGHAN become available, the contribution of an expert gastrointestinal pathologist is extremely important.

Imaging Studies (Figs. 27.6, 27.7, 27.8, 27.9, 27.10, 27.11, and 27.12) [52, 53]

In patients with mucosal disease, imaging studies of the GI tract may reveal thickening or nodularity in the antrum and thickening or "saw-tooth" appearance of the mucosa of the small bowel [56]. It should be noted, however, that the above findings lack of sensitivity and specificity regarding EGE.

Imaging studies are particularly important tools for the diagnostic approach of muscular and subserosal subtype of EGIDs. Barium follow through (Figs. 27.6 and 27.7) [52, 53], abdominal ultrasound, abdominal computed tomography scan (Figs. 27.8 and 27.9) [51], or magnetic resonance imaging (Figs. 27.9 and 27.10) [51] may reveal in patients with muscular involvement, irregular narrowing of the lumen, especially in the area of the distal antrum and proximal small bowel [52, 53, 74].

Other Tests

In patients with subserosal disease and ascites, increased eosinophil counts have been reported in the ascitic fluid analysis (Fig. 27.12) [61]. The analysis of the ascitic fluid apart from cytology, it should also include Gram stain, acid-fast bacillus stain, fungal cultures, and mycobacterial cultures. Studies have documented a marked elevation of the eosinophil counts up to 88% in patients with subserosal subtype of EGE [23]. It should be noted, however, that no strict criteria to evaluate the eosinophilia of the ascitic fluid are available.

Differential Diagnosis (Table 27.2)

The differential diagnosis of EGIDs includes infections, allergic conditions (such as drug allergy), hypereosinophilic syndrome, inflammatory bowel diseases, autoimmune diseases, connective tissue disorders, malignancy, chronic graft versus host disease, immunodysregulation, etc. [17, 35, 40, 59], while the differential diagnosis of EC should also include mastocytic enterocolitis [75, 76] or systemic mastocytosis [77, 78].

To exclude parasitic infections, it is necessary to check stool microscopy for ova and parasites and serology for *Strongyloides* and *Toxocara* species, while patients with a recent history of travel to (or residence in) endemic areas should be tested also, for antibodies to fungi and parasites such as *Coccidioides, Echinococcus, Schistosoma,* and *Trichinella spiralis.*

Allergic colitis of infancy may also be associated with >20 eos/hpf in rectal biopsies [79], with patchy distribution [80], but the disease resolves clinically and histologically following the removal of the offending antigens (typically

cow's milk) from the diet of the affecting infant. Tissue eosinophilia can also be found in colon biopsies of patients in immunosuppression following transplantation, especially in those receiving tacrolimus [81–83].

Shoda et al. [32] tried recently to uncover likely molecular pathogenesis that accounts for the distinct endoscopic and histologic features of EG and developed tissue- and blood-based platforms for its diagnosing and monitoring. They enrolled 185 patients (74 patients with EG and 111 without) across nine Consortium of Eosinophilic Gastrointestinal Disease Researchers–associated sites. The researchers analyzed an EG Diagnostic Panel (EGDP; gastric transcript subset) and an EG blood biomarker panel (protein multiplex array). EGDP scores were derived from the expression of 18 highly dysregulated genes, while blood EG scores were derived from dysregulated cytokine/chemokine levels. The authors reported that the EGDP18 scores were inversely correlated with gastric peak eosinophil counts, periglandular circumferential collars, and endoscopic nodularity. With regard to blood-based platforms, the authors [32] reported significant increases in eotaxin-3, thymus, and activation-regulated chemokine, IL-5 levels, as well as thymic stromal lymphopoietin levels. Blood EG scores were able to distinguish patients with EG from control subjects: They positively correlated with gastric eosinophil levels and inversely correlated with plasma and serum EGDP18 scores. The authors concluded that EGDP scoring based on tissue- and blood-based platforms is an important diagnostic tool for assessing EG. Further studies are required for the validation of the above platforms, assessment of their efficacy, and optimization for disease stratification.

Treatment

Due to the rarity of EGIDs beyond the esophagus, there are no randomized controlled studies regarding standardized treatment options in children [17, 40]. Most evidence is based on case reports and case series and few randomized trials in adults. Corticosteroids are a mainstay of treatment for inducing remission, but there may be a subset of patients in whom elimination diet can be effective. In some occasions, the disease is not easily controlled [40, 48], while maintenance therapy is often required [29, 48, 67]. Additional therapies have been reported in case reports or small series of patients [84] with conflicting results, while novel drugs [85, 86] for treating refractory disease are currently under investigation.

Table 27.2 Differential diagnosis of eosinophilic gastrointestinal disorders beyond eosinophilic esophagitis

EG	Helicobacter pylori infection Inflammatory bowel disease Connective tissue disorders Hypereosinophilic syndrome
EGE/EC	Infections (parasitic, amebic, fungal) Inflammatory bowel disease Connective tissue disorders Hypereosinophilic syndrome Vasculitis Malignancy

EG Eosinophilic Gastritis, *EGE* Eosinophilic Gastroenteritis, *EC* Eosinophilic Colitis

(i) *Dietary Treatment*

In some pediatric patients with EGIDs beyond the esophagus, symptoms and tissue accumulation of eosinophils may be reduced by antigen restriction [3]. Ko et al. evaluated, retrospectively, the response to dietary treatment of 30 children with EG, 43% of whom had concomitant EoE and 21% EGE [3]. The dietary therapy included elemental diet, seven food elimination diet (milk, egg, wheat, soy, peanut/tree nuts, fish/shellfish, and red meet) or empiric avoidance of one to three foods. Overall, 82% of patients achieved clinical remission. However, histological assessment was available in only up to five children per each dietary treatment group, 78% of whom achieved histological remission, making difficult to draw conclusions on efficacy [3]. Patients with concomitant esophageal eosinophilia appeared to be more resistant to dietary therapy since 6 of 16 patients were noted to have persistent esophageal eosinophilia despite resolution of findings in gastric biopsies. The above study showed also no correlation between response to dietary therapy and food sensitization, despite the fact that 86% of patients were found to be sensitized to several foods using skin prick tests or serum analyses [3].

In patients with EGE and symptoms of malabsorption, an initial therapeutic approach could involve an empiric six-food elimination diet or an elemental diet. Elemental diet eliminates all potential food allergens and has been reported to be beneficial in selected patients [28, 87]. Six-food elimination diet, excluding soy, wheat, egg, milk, peanut/tree nuts, and fish/shellfish, foods that most commonly cause hypersensitivity reaction in the general population, is the most commonly used diet, empirically, in such patients [88]. Reed et al. [48] reported that in 21 of 44 patients with EGE (13 being placed on an elemental formula, seven on a testing directed diet, and one not specified) 12 patients (57%) had resolution of symptoms. However, 15 of them received concomitant corticosteroids while histological response was not assessed [48]. Gonsalves et al. carried out a prospective study published recently as Conference abstract [30], assessing the efficacy of elemental diet in 15 adults with EG/EGE, 87% of whom atopic comorbidities. The authors reported that elemental diet for 6 weeks was associated with histological remission in all patients, significant improvements in clinical symptoms, endoscopic findings, in depression and fatigue domains of the patient reported outcomes' information measurement scores and also of the EGDP score [30]. A

systematic review carried out by Lucendo et al. [88] assessed the efficacy of dietary interventions in children and adults with EGE. The review included 30 studies, most of which were of low quality. Elemental diets were associated with clinical improvement in 75% of patients, but histological assessment was carried out in only six patients, five of which had achieved histological remission. Empiric elimination diets (6FED or 7FED that included the elimination also of red meat) were introduced to 34 patients and were associated with clinical improvement in 85% of them (histological response was not assessed), while single food (cow's milk) elimination diet was introduced to 16 patients, 62% of whom improved clinically, without though, assessing histological response. In patients who were assessed for histological response, a significant reduction in gastric (but not duodenal) eosinophilic infiltration was reported [88]. More recently, a retrospective multicenter study [89] reported on treatment response of 109 patients with EGIDs beyond EoE who had a 6-month follow-up from the start of treatment. The authors reported that all types of elimination diets (6FED, elemental and targeted diet) were associated with clinical improvement. Furthermore, patients with EG showed a significant reduction in eosinophilic gastric infiltration, while those with EGE showed a significant decrease in gastric and a nonsignificant in duodenal eosinophilic infiltration [89].

Dietary therapy should be under the guidance and supervision of a dietician trained in EGIDs in order to assure the patient's compliance and to avoid nutritional compromise. In patients with EGE and peripheral eosinophilia, a reduction of >50% could be considered as a response to dietary therapy. In all cases of uncertainty regarding the response to treatment and/or the degree of activity of the ongoing disease, a repeat endoscopy with biopsies should be performed. Once the dietary intervention is successful at reducing symptoms, peripheral and/or tissue eosinophilia, foods are reintroduced slowly, starting from the least allergenic to most allergenic.

To conclude, some studies suggest that dietary treatment may be an alternative for inducing remission in patients with EGIDs beyond EoE, but data on histological response and on the long-term disease outcome are scarce, the possible food triggers have not been identified and the process of food reintroduction is not clear. Furthermore, although food allergens' hypersensitivity plays an important role in the pathogenesis of EGIDs, there is no evidence to support the routine use of food allergy testing, in making clinical decision.

(ii) *Drug Treatment*

Corticosteroids

Oral Corticosteroids

Corticosteroids are known to decrease the recruitment of inflammatory cells including eosinophils, to decrease the release of eotaxins and other inflammatory mediators, and to reduce capillary permeability. Pineton et al. demonstrated efficacy of oral corticosteroids in 18 out of 19 (95%) patients with EGE in whom it was used among a total number of 43 patients [67].

As fibrosis in EGE is much less common in EGE compared to EoE, the minimum dose of oral corticosteroids can improve EGE symptoms and control peripheral eosinophilia, avoiding the systemic side effects associated with high doses. Improvement in symptoms with the use of corticosteroids usually occurs within 2 weeks regardless of the layer which is involved [46, 47]. Prednisone is introduced at a dose of 1–1.5 mg/kg for 2 weeks, and subsequently, it is tapered rapidly over the next 2 to 4 weeks.

Topical Corticosteroids

Case reports have indicated that fluticasone improves gastric eosinophilia when used primarily to treat EoE [90], while others reported that the off-label use of formulations of budesonide in form of capsules, dissolved in water, may target the upper GI tract [59]. Treatment with topical steroids in the form of budesonide, as a budesonide capsule, has also been reported to be occasionally effective in patients with EGE involving the antrum and the small intestine [91–94].

In some circumstances, more prolonged therapy with glucocorticoids is needed in order to succeed complete resolution of symptoms [46, 95]. Patients who do not respond to oral glucocorticoids should undergo a course of equivalent intravenous glucocorticoids and in case of failure, a thorough reevaluation is needed in order to rule out other possible underlying conditions.

Cromolyn, Ketotifen, and Montelukast

Cromolyn is expected to prevent the release of mast cell mediators such as histamine, platelet-activating factor, and leukotrienes and reduce antigen absorption by the small intestine. Some case reports have documented its effectiveness for short- and long-term management of EGE, while others not [9, 96, 97]. Ketotifen is an H1-antihistamine and mast cell stabilizer which has been reported to improve clinical symptoms and tissue eosinophilia in small series of patients [98–100]. Montelukast is a leukotriene antagonist which has been reported to be effective in some case reports but not in others [64, 101–104].

Reed et al. assessed the efficacy of various treatment options including dietary therapy, corticosteroids, mast cell inhibitors, H_2 antagonists, and leukotriene receptor antagonists in 44 patients with EGE, including pediatric patients, for an average of 26.2 months, 76% of whom needing more than one treatment option [48]. None of the patients treated only with mast cells inhibitors, leukotriene receptor antagonists, or H_2 antagonists achieved clinical or histological remission [48]. When all treatment modalities were considered, 60% of patients presented resolution of symptoms and

51%, histological remission. Corticosteroids appeared to be the most effective treatment achieving resolution of symptoms in 22/36 (61%) patients, while dietary therapy in 12/21 (57%) patients, 71% of whom received also treatment with corticosteroids. Twenty-eight patients were assessed for histological response, 19 of whom demonstrated remission after treatment: 9/19 had received corticosteroids, 6/19 combination treatment with corticosteroids and dietary therapy, and 4/19 only dietary therapy [48].

In summary, although studies to date do not advocate the use of leukotriene receptor antagonists, cromolyn, or ketotifen as monotherapy in patients with EGIDs, in case of existing comorbidities for which they are indicated, evidence suggests that they are unlikely to be harmful.

Biological Agents and Immunosuppressive Drugs

Humanized anti-IL-5 antibody has been reported in a preliminary report of a small number of patients to reduce peripheral and tissue eosinophilia without improving symptoms [105, 106], while after the discontinuation of the treatment eosinophilia reappeared. Omalizumab, an anti-IgE monoclonal antibody, was reported in nine patients to be associated with improvement in symptoms and measures of IgE-mediated allergy, decrease in peripheral eosinophilia but no reduction in tissue eosinophilia [10], thus has no place in the treatment of EGIDs. In a recent study [107] on mouse model of food allergen induced GI eosinophilic inflammation, anti-CCR3 antibody significantly reduced the severity of eosinophilic inflammation, mucosal injury, and diarrhea. CCR3 may be a novel therapeutic target for treatment of EGE and other GI eosinophil-mediated diseases, but further studies are necessary to determine whether the above results can be extrapolated to humans. Suplatast tosilate, a novel antiallergic drug that seems to suppress the production of cytokines, including interleukin (IL)-4 and IL-5 from T helper 2 (Th2) cells, was reported to be effective in a single patient [108]. Thiopurines, such as azathioprine, have been reported to be effective in refractory cases [109]. A recent study [85] using an anti-integrin agent (Vedolizumab) in adult patients with steroid refractory EGE reported clinical and histological improvement in three-fourths of steroid-refractory patients receiving Vedolizumab, which is quite promising but more studies in children are necessary.

Another agent that also seems promising is Siglec-8 blocker that was assessed recently in a double-blind, placebo-controlled trial [86] in 65 patients with refractory EGE (AK002: 43, placebo: 22). The mean percentage change in gastrointestinal eosinophil count was -86% in the combined AK002 group, as compared with 9% in the placebo group. Treatment response occurred in 63% of the patients who received AK002 and in 5% of the patients who received placebo. The mean change in total symptom score was -48% with AK002 and -22% with placebo. Adverse events associated with AK002 were similar to those with placebo, with the exception of higher percentages of patients having mild-to-moderate infusion-related reactions with AK002 (60% in the combined AK002 group and 23% in the placebo group) [86].

In conclusion, although several novel drugs seem quite promising in treating EGIDs, more trials are required in adults and in children with EGIDs to assess their efficacy and safety. Due to limited number of randomized controlled trials on the efficacy of the above drugs, they cannot be recommended for routine use in pediatric patients with EGIDs, but they can be used in EGID centers either as part of research studies or for selected patients with refractory disease. Well-designed randomized controlled trials are urgently needed to establish the best drug treatment option for refractory disease.

(iii) *Surgical Treatment*

Surgical treatment is limited only to highly selected cases of persistent pyloric or small bowel obstruction with increased percentage of recurrence [110, 111].

Natural History of EGIDs Beyond EoE

Unfortunately, only few patients with EGE present complete long-term remission with treatment. In most patients, the disease is chronic, relapsing, with periodic flares months to years after the initial episode if long-term maintenance treatment is not applied.

Chen et al. noted that among 15 patients with EGE, 13 patients treated with oral corticosteroids, followed by a rapid tapering, manifested symptom remission within 2 weeks but five of them relapsed within 12 months from drug discontinuation [29]. Patients who have recurrent symptoms with periodic flares months to years after the first episode may undergo another short course of oral glucocorticoids, typically prednisone, followed by a rapid taper [46]. Pineton et al. assessed the long-term outcomes of 43 patients with EGIDs and noted that 18 of them (42%) had an initial flare without a relapse, 17 (37%) presented recurrent disease with multiple flares among periods of full remission and 9 (21%) had chronic disease. Among patients with recurring disease, an important variability of the intervals between flares was observed, ranging from months to years [67]. Similarly, Reed et al. noted that only one-third of the pediatric and adult EGE patients, enrolled in their study, underwent long-term remission [48].

More studies are needed to better understand the pathogenesis and the natural history of various disease subtypes, their impact on patient's growth, development and quality of life, and the effectiveness of various therapeutic approaches. Understanding this variance appears to be very important in educating patients and analyzing the outcomes of different

therapeutic options, especially in patients who present refractory disease [32]. With all subtypes of EGIDs increasing more and more, emerging phenotypic data may facilitate the understanding of the complex pathogenetic mechanisms behind the disease, leading to an individualized short- and long-term management. Furthermore, international, consensus recommendations on diagnostic criteria for the diagnosis of the EGIDs beyond EoE are urgently needed to facilitate well-designed, randomized trials assessing the efficacy of various treatment modalities. ESPGHAN and NASPGHAN are about to produce such document to encourage high quality research, and to guide clinicians dealing with these disorders, decreasing variations in clinical practice and improving effectiveness and quality of care.

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Crohn's Disease

28

Marina Aloi and Salvatore Cucchiara

Introduction

Crohn's disease (CD) is a chronic, relapsing disorder of the gastrointestinal tract, belonging to the inflammatory bowel diseases (IBDs). Approximately 25% of IBD is present in childhood and adolescence, with recent studies suggesting that the prevalence is rising in both developed and developing countries [1–4]. The precise cause of IBD is still unsettled, but there is evidence that they develop as a consequence of an abnormal immune response to the intestinal microbiota in a genetically susceptible host [5, 6]. Although pediatric- and adult-onset IBD seem to share many clinical aspects and pathogenetic pathways, however, some features characterize the pediatric form, such as the potential for linear growth impairment and pubertal delay, as complications of undertreated inflammation and malnutrition, the influence of nutritional treatment on the course, and the different phenotype expressions of the disease [7]. Finally, evidences from animal models of IBD and preliminary observations in children support the concept that IBD develops in distinct phases and that key mediators of the inflammation may play different roles, depending on the stage of the disease [8, 9]. These observations highlight the importance of taking into consideration the disease course when studying pediatric IBD and targeting appropriate treatments.

Epidemiology

Although IBD can occur at any age, up to 25% of patients develop symptoms during childhood and adolescence [1–3]. Some epidemiological studies from the United States and Europe have shown a steady increase in the overall mean annual incidence of IBD around the world: the rising rate of pediatric IBD seems to be primarily due to an increase of the incidence of CD [1, 3, 10]. CD is unequally distributed all over the world, with highest rates occurring in Western and Northern countries, and with a decreasing gradient from North to South and from West to East [4]. The worldwide highest prevalence of pediatric IBD is reported from the Canadian Ontario region, with approximately 50 IBD patients per 100,000 inhabitants [3]. Recent epidemiological observations from two large claims databases in the United States reported an increased prevalence of IBD overall by 133%, from 33/100,000 in 2007 to 77/100,000 in 2016. Among children, CD was twice as prevalent as UC (45.9 vs 21.6). Prevalence was higher in boys than girls for all forms of IBD, in contrast to the adult population where the prevalence was higher in women than men [1]. In Europe, a recent study based on the Epidemiology of Inflammatory Bowel Diseases (EPIMAD) data of Northern France indicated a dramatic increase of both CD and UC in adolescents from 1988–1990 to 2009–2011: for CD, from 4.2 to 9.5/105 (+126%) and for UC, from 1.6 to 4.1/105 (+156%) [10]. Factors contributing to the evident increase in the global incidence could be a greater case ascertainment, the widening case definition, earlier onset in predisposed individuals, and greater access to health care; however, it is widely agreed that the rising incidence of pediatric IBD is due to a real increase in the number of affected children [11]. It has been postulated that the "Westernization" of different societies accounts for the progressive rise in the incidence of the disease also in previously low-incidence areas, including Japan [12], other Asian countries [13, 14], and some Eastern European countries [15].

Pediatric IBD present specific phenotypic and demographic differences when compared with the adult-onset disease. While CD and UC occur with an equal distribution in adults [4], it has been reported that in childhood CD is more frequently diagnosed than UC [1]; moreover, while in adults there is an equal male-to-female ratio (or a mild female

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predominance), all pediatric IBD cohort studies or registries indicate a male predominance [16]. In pediatric CD, most patients have an extensive disease, ileocolonic or colonic, and distinction of UC from colonic CD may not be uncommonly challenging [17]. Moreover, children with CD are more likely to have upper gastrointestinal involvement than their adult peers [18]. Pediatric CD also seems to have a different behavior than the adult-onset disease: children often present with an inflammatory or nonstricturing, nonpenetrating disease, while complicated disease is fairly unusual at presentation. However, even with treatment, many data demonstrate that inflammatory CD progresses to stricturing and penetrating disease in several children [18, 19]. Adult disease begins more often with a complicated behavior (stricturing or penetrating), with a lower trend of disease progression [20].

Etiopathogenesis

The most accepted hypothesis for the pathogenesis of CD is that the interaction between luminal contents (i.e., the intestinal microbiota) and the mucosa leads to a dysregulated inflammation in a genetically predisposed host. Several microorganisms have been historically considered as potential causative agents for CD, including *Mycobacterium paratuberculosis*, *Listeria monocytogenes*, novel Burkholderiales, and *Escherichia coli* [21, 22]. Interestingly, strains of adherent-invasive *Escherichia coli* (AIEC), capable of adhering to and invading epithelium, and to replicate in macrophages, have been described in adults and children with CD [23–25]. Nevertheless, there are no strong data to support a role for any of these microorganisms as the causative factor in the etiology of IBD. Some interesting findings in the pathogenesis of IBD come from genetics. The importance of genetics in CD was suggested by family, twin, and phenotype concordance studies. Monozygotic twins exhibit phenotypic concordance in 50–70% of CD patients, and their relative risk of developing CD is 800-fold greater compared to the general population [26–28]. Recently, the discovery of several susceptibility genes has further secured the importance of genetic predisposition in CD and UC [26]. After the pivotal study of Hugot et al., in 2001, who discovered the association of variants of the NOD2 gene with ileal CD [29], and the discovery of the correlation between variants of IL23 receptor (IL23R) gene and both CD and UC in 2006 [30], the number of IBD genetic associations is dramatically increased. More susceptibility loci have been quickly identified, such as autophagy genes, ATG16L1 and IRGM [31, 32]. In the last decade, the implementation of genome-wide association studies (GWAS) has significantly advanced our knowledge on the importance of genetic susceptibility in IBD [33]. To date, GWAS have identified more than 200 risk-conferring loci for IBD [34], most of them shared by CD and UC. However, some genes are quite different for CD or UC, clearly denoting the genetic heterogeneity of the two forms of IBD, each one of them showing distinctive and shared genetic associations. For instance, NOD2 and autophagy genes, and ITLN1 (Intelectin 1) are unique for CD [34]. Despite the discovery of a massive number of susceptibility genes for IBD, we are still far from understanding the mechanism by which such genetic variants cause the uncontrolled intestinal inflammation. The challenge for basic IBD researchers is now to identify how genetic abnormalities influence pro-inflammatory pathways, providing information directly improving the clinical management. Some pro-inflammatory pathways have been partially or totally understood, in some cases this knowledge has enabled the development of specific interventions. For instance, NOD2 gene defected patients have an impaired ability to recognize and process bacterial products, and this may lead to an inappropriately innate immune response. Some CD patients with variants of the autophagy genes (ATG16L1 and IRGM) have a defective capacity to process cell degradation products, as well as bacteria, and therefore an insufficient ability to eliminate pro-inflammatory factors [23]. One of the most important discoveries in the field of genetics of pediatric IBD is the identification of impaired interleukin 10 (IL-10) signaling in some forms of very early onset (within the first months of life) CD [35]. The common characteristics of these patients are a very early onset of a severe form of CD (infantile colitis), with colonic involvement and presence of fistulae, in some patients associated with growth failure, resistant to conventional therapies. Allogeneic hematopoietic stem cell transplantation is effective in most patients [36]. This form of CD is due to homozygous mutations in either IL10RA or IL10RB, which encode subunits of the IL10 receptor, or for IL10 itself [37]. One could speculate that this form of IBD with a monogenetic inheritance could identify a subset of patients with a "more" Mendelian transmission, opening new horizons for research and also expectations to understand the definite mechanisms underlying these diseases. Since then, several other forms of monogenic chronic inflammatory conditions with an IBD phenotype have been discovered, sharing a disturbance of the intestinal epithelial barrier function or a dysfunction of the innate and adaptive immune system, with a wide spectrum of disease presentations and with different courses and medical and surgical treatments, although allogeneic hematopoietic stem cell transplantation is often the only effective therapy [36, 38, 39]. A correct diagnostic approach, including genetic analysis, is mandatory in children who develop a very early onset IBD, and possibly in general in those patients with a severe CD course [36].

Beyond the genetics, in the last two decades epidemiological and pathogenetic studies have been mainly focused on the role of environmental factors in the etiology of IBD. Indeed, the epidemiology of both CD and UC clearly

addresses environment as the main factor that can determine such rapid changes. Moreover, the increasing incidence and prevalence of pediatric IBD worldwide, both in developed and in developing countries, seem to follow changes in the diet and lifestyle, with the adoption of a "Westernized" lifestyle [5]. Even though a causative role for a single factor has not been proven, several potential environmental influences have been studied so far. Smoking has been widely demonstrated to negatively affect the risk of developing CD and to exacerbate its disease course. The extensive use of antibiotics and gastrointestinal infections, particularly in the early ages of life, seem to increase the risk of developing IBD. Personal stress is recognized as a risk factor for disease exacerbations as well [40]. Among all, the spread of the "Western" diet, high in fat and protein but low in fruits and vegetables, is regarded by many researchers as a strong candidate, and its influence on gut inflammation is highly hypothesized [41].

Clinical Presentation

CD is characterized by transmural inflammation that can be found everywhere in the gastrointestinal tract, from mouth to anus. The terminal ileum is the most common site of CD, however about 60% of children have an extensive ileocolonic involvement, and 20–30% an isolated colonic disease [17]. CD typically presents in any age group with a constellation of abdominal pain, diarrhea, weight loss, and poor appetite, however, short stature and predominant perianal disease are further significant features of presentation of pediatric CD. Impairment of linear growth and associated delay in sexual development can present before the onset of intestinal symptoms and can dominate the clinical presentation [42]. Growth failure is a unique characteristic of pediatric-onset CD: it is defined as linear growth at or <2 SD below the mean for age, or decreased growth velocity, and can occur in 15–20% of children with CD. The onset of growth failure is usually insidious, and any child or adolescent with impairment of linear growth should have an appropriate initial diagnostic evaluation for IBD. The assessment of growth and pubertal delay is a key factor in the management of pediatric IBD. Maintaining adequate nutrition, minimizing inflammation, and maximizing corticosteroid-free treatment remain a crucial part of managing the potential growth stunting effects of active IBD, most specifically of small bowel CD. During the clinical course of CD, growth failure has been reported in up to 40% of children with CD and a final height below the fifth centile is reported in 7–30% of patients with pediatric-onset CD [43]. Growth failure in CD originates from different factors, such as malnutrition with decreased intake, increased gastrointestinal losses, malabsorption, and medication effects. All these components might certainly impact an individual's nutritional state. Nevertheless, it is widely agreed that the inflammation process per se may directly inhibit linear growth and play a major role in the etiology of growth retardation. Inflammatory mediators, such as interleukin 6 (IL-6) and tumor necrosis factor (TNF)-alpha, are key factors in reducing plasma levels of insulin-like growth factor 1 (IGF-1), the peripheral mediator of the growth hormone [44]. Impressive catch-up growth can be observed as soon as remission of the intestinal inflammation is achieved. It is essential that height, weight, puberty staging, and bone age are accurately and regularly measured and recorded in young patients with CD. Nutritional supplementation and need for "catch-up" growth should be part of the evaluation of a CD pediatric patient. Delay of pubertal onset is also common in active pediatric CD, and the duration of puberty can be impaired too. Active or relapsing disease during the years following the onset of puberty may slow or even arrest the progression of puberty. Moreover, pubertal delay affects estrogen and androgen levels, which are critical for normal bone mineralization, contributing to the development of osteoporosis and osteopenia. Inducing and maintaining disease remission before and during the pubertal years is crucial in order to ensure the attainment of final predicted height, avoiding a missed pubertal growth spurt.

Diagnosis

There is no gold standard single test allowing a definite diagnosis of CD; however, the combination of family and clinical history, physical examination, and subsequent laboratory, imaging, and endoscopic assessment usually leads to the correct diagnosis. Nevertheless, 10–15% of children with IBD confined to the colon receive an initial diagnosis of inflammatory bowel disease-unclassified (IBD-U) and they will be subcategorized as CD or UC only during follow-up [45]. The first diagnostic issue is whether the presenting symptoms are related to CD. When the classic clinical presentation is evident, differentiating CD from other diseases with initially similar symptoms may be relatively simple [46]. However, in a child with bloody diarrhea, infectious etiologies by invasive agents, like *Salmonella*, *Yersinia*, *Shigella*, *E. coli*, etc., must be excluded. It is also important to evaluate and rule out vasculitides (such as Henoch–Schonlein purpura and hemolytic–uremic syndrome) or allergic colitis. An abdominal pain mimicking CD pain may be determined by other conditions, such as lymphoma, ovarian cyst, appendicitis, or intussusceptions. Figure 28.1 shows the suggested diagnostic work-up of children or adolescents with suspected IBD.

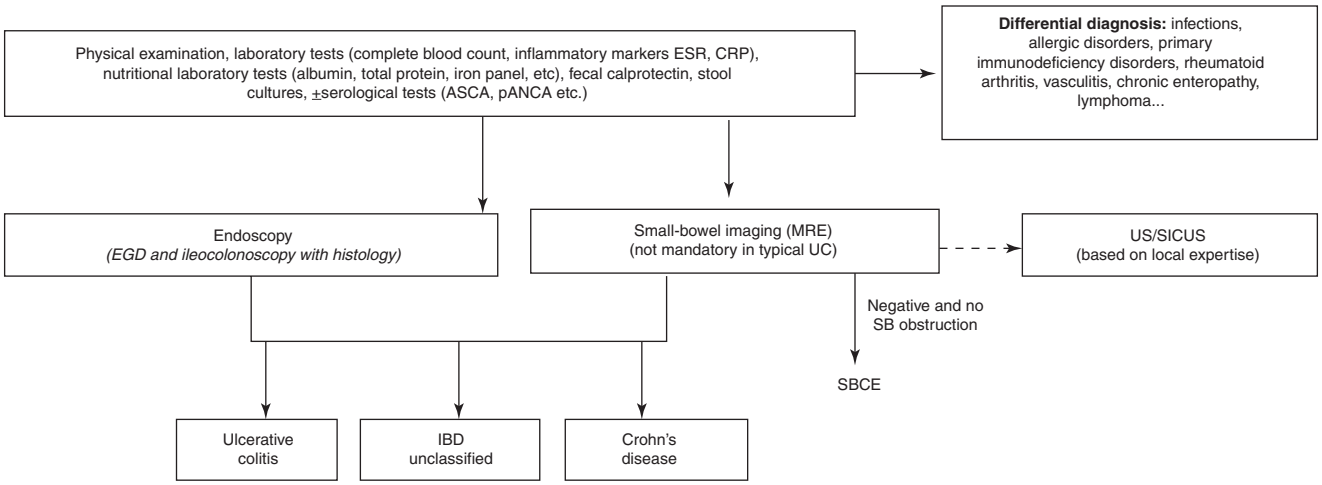

Fig. 28.1 Diagnostic work-up of children or adolescents with suspected IBD. *ESR* erithrocyte sedimentation rate, *CRP C*-reactive protein, *ASCA* anti-Saccharomyces cerevisiae antibodies, *pANCA* perinuclear antineutrophil cytoplasmic autoantibodies, *EGD* esophagogastroduodenoscopy, *MRE* magnetic resonance enterography, US abdominal ultrasound, *SICUS* small-intestine contrast ultrasonography, *SBCE* small-bowel capsule endoscopy, IBD inflammatory bowel disease

Noninvasive Tests

Serologic Tests

Serologic tests are the first diagnostic tool for suspected CD. They mainly include complete blood count and acute phase reactants (i.e., C-reactive protein, erythrocyte sedimentation rate, ferritin, platelet count). C-reactive protein (CRP) is a marker of inflammation, and its concentration reflects disease activity in patients with CD. However, CRP is nonspecific, particularly in children, since its serum levels can raise because of other inflammatory conditions beyond the gut, while genetic factors, along with age, sex, and body mass index (BMI), may influence CRP production. Nevertheless, there are no alternative biomarkers for use in routine clinical practice [47, 48]. Due to the transmural inflammation, CRP plays a major role in CD monitoring: indeed, previous studies have shown that patients treated with infliximab and early normalization of CRP levels were more likely to maintain response to treatment, while persistently abnormal CRP levels correlated with higher relapse risk [49, 50]. The optimal CRP cut-off level for monitoring disease activity is unclear: in a large multicenter study, CRP > 5 mg/L (CRP > 0.5 mg/dL) was a criterion for treatment failure [51].

Several antibodies to microbial antigens have been identified in patients with IBD. The most extensively studied and commonly used are anti-*Saccharomyces cerevisiae* antibodies (ASCA) and perinuclear antineutrophil cytoplasmic autoantibodies (pANCA). ASCAs have initially been suggested as a marker for CD with a prevalence of 50–60%, compared with 10–15% in UC and 0–5% in healthy controls [52, 53]. Conversely, pANCAs have been proposed as a marker for UC or colonic CD [54]. However, between 30% and 50% of the IBD patients did not screen positively for either of these markers, so they could not be used for diagnostic purpose, except in the context of an IBD-U, where ASCA or pANCA positivity might help labeling the patient as a CD or UC, respectively [55, 56]. The attention of researchers has been recently focused on the possible relationship between serologic markers and distinct subgroups of patients and prognosis of the disease [57]. In CD, positive ASCA titers have been correlated to younger age at onset, ileal disease, severe behavior, and increased risk of early surgery [57]. Because of these promising data, other markers useful for prognostic purposes have been identified. Reactivity to *Pseudomonas fluorescens*-related protein was independently related to stenosing disease and need for surgery, whereas anti-*Escherichia coli* outer membrane porin C (OmpC) has been associated with penetrating disease [57]. Both the presence and magnitude of the immune response seem to be correlated with more aggressive disease courses. Recently, a large, multicenter, prospective study tried to derive a risk-stratification model of a complicated behavior based on clinical and serologic factors defined at diagnosis in pediatric CD. Stricturing disease was associated with ASCA, antibody reactivity to bacterial flagellin (CBir1), and neutralizing auto-antibodies (Ab) against granulocyte-macrophage colony-stimulating factor antibody (GM-CSF Ab), penetrating disease was associated with older age, African American race, and ASCA and CBir1 seropositivity in univariate analyses. The authors also found that *Ruminococcus* was implicated in stricturing behavior and *Veillonella* in penetrating complications. Moreover, ileal genes controlling extracellular matrix production were associated with stricturing complications [8].

Fecal Markers of Inflammation

The neutrophil-derived marker calprotectin is a noninvasive tool for the diagnosis and monitoring of the activity of IBD. Calprotectin is a calcium-binding protein that is excreted in the feces and can be readily measured using an ELISA (enzyme-linked immunosorbent assay). The protein is stable in stool specimens for up to a week at room temperature, allowing patients to collect a specimen at home without special precautions. Calprotectin is commonly used in the initial diagnostic approach to suspected IBD: a fecal calprotectin concentration of less than 40 μg/g in the presence of abdominal symptoms, suggestive of irritable bowel syndrome, is associated with only 1% chance of IBD, therefore reducing the number of negative endoscopies in both children and adults. Conversely, higher fecal calprotectin values (a cut-off of 100 μg/g has a good sensitivity for discriminating patients with suspected inflammation) help in identifying those patients who need investigation (i.e., endoscopy) and are most likely to have IBD [58].

Fecal calprotectin is, moreover, equally useful in the context of disease monitoring, due to its ability to confirm tissue healing and predict disease relapses. A few studies have evaluated this outcome, demonstrating a sensitivity of 89–90% and specificity of 82–83% for predicting disease relapse during a 12-month period, with a sensitivity and specificity to predict the absence of mucosal healing of 70–100% and 44–100%, respectively, depending on the calprotectin concentration threshold used [59]. Other fecal biomarkers, such as lactoferrin, neutrophil-derived S100A12 and High-Motility Group Box 1 (HMGB1), have shown promises as potential biomarkers for CD, but have been studied far less extensively [60, 61].

Ileocolonoscopy and Esophagogastroduodenoscopy (EGD)

Traditional endoscopy has a pivotal role in the diagnosis of suspected CD. According to the last European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines on the diagnosis of IBD, ileocolonoscopy and EGD should be recommended as the initial work-up for all children with suspected disease. Multiple biopsies should be obtained from all sections of the examined gastrointestinal tract, even in the absence of macroscopic lesions [46]. Beyond diagnostic aims, traditional endoscopy has an important role in staging disease severity, evaluating and treating strictures, detecting postoperative recurrences, surveilling neoplasms, and preoperative assessment. Moreover, endoscopy allows the monitoring of response to therapies, by evaluating mucosal healing (MH). In the last decade, MH has become the most rigorous endpoint in both adult and pediatric therapeutic trials. The so-called treat-to-target strategy for disease management and the importance of reaching MH will be further discussed at the end of this chapter. Tables 28.1 and 28.2 show the typical endoscopic and histologic findings differentiating CD and UC.

Table 28.1 Endoscopic differentiation between typical CD and UC

CD	UC
Throughout the entire GI tract	Confined to colon
Discontinuous lesions	Usually continuous lesions
Rectal sparing or segmental inflammation	Rectal involvement (in children may be absent)
Aphthous ulcers (may occur in normal mucosa)	Mucosal granularity/friability
Linear ulcers common	Erosions/microulcers
Cobblestoning	Loss of vascular pattern
Ileocecal valve stenotic and ulcerated	Ileocecal valve patulous and free from ulcerations (possible backwash ileitis)

Table 28.2 Histologic findings in CD and UC

CD	UC
Focal crypt distortion Ulcers and/or aphthoid ulcers Mucin depletion absent or weak Pseudopyloric metaplasia Focal cryptitis Focal lympho-plasmacellular infiltration in the lamina propria Granulation tissue-like inflammation Epithelioid granulomas	Mucosal surface alteration Crypt distortion Atrophy Mucin depletion Cryptitis and/or crypt abscesses. Diffuse lympho-plasmacellular infiltration in the lamina propria Basal plasma cell infiltration

Small Bowel Evaluation

Imaging

Most CD patients, particularly those with a childhood-onset disease, present with a small bowel involvement. The revised PORTO criteria state the need of small bowel assessment at the diagnosis in all suspected CD cases, through magnetic resonance enterography (MRE) or wireless capsule endoscopy [62]. Furthermore, children with CD need frequent evaluations during the course of their disease, so noninvasive, radiation-free imaging tests, could be a valuable alternative to endoscopy for the definition of activity and complications of the disease. In the past, small bowel follow through (SBFT) was the "gold standard" for small bowel disease, but it no longer plays any role both in CD diagnosis and follow-up. In the last decade, due to considerable advances in technologies, other modalities, such as MRE, ultrasound (US), and computed tomography (CT), have taken its place [63, 64]. Computed tomography has the major disadvantage of the large radiation exposure, therefore limiting its use only to the

emergency setting, when magnetic resonance (MR) is not available. Magnetic resonance with the administration of oral contrast (MRE) results in luminal distension, facilitating evaluation of bowel wall thickness, profile, and structure; MR enteroclysis (with contrast administered through a nasojejunal tube) seems to have the same sensitivity (88%) of MRE but a superior patient discomfort [63], and therefore it is not recommended in children with CD. In comparison with endoscopy, MRE has a sensitivity of 84–96% and a specificity of 92–100% for the diagnosis of IBD [65]. MRE can also allow a detailed assessment of perianal fistulae, and it should be preferred as the imaging tool for perianal disease [66]. The last European Crohn's and Colitis Organization (ECCO) and the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines on the management of pediatric CD recommend MRE as the imaging tool of choice for the diagnosis and follow-up of CD [67]. Abdominal US is a widely used technique in the evaluation of patients with CD because of its excellent safety profile, low cost, and recent advances in the equipment (Doppler and use of oral contrast) that allow high-resolution images [64, 68]. Recently, small intestine contrast ultrasonography (SICUS), performed after ingestion of an oral contrast material filling the small bowel lumen with anechoic fluid, has been developed for the study of the small bowel [68]. SICUS provides the opportunity to visualize and assess the entire small bowel by measuring the intestinal wall thickness and lumen diameter at different levels [63]. Ultrasound techniques have several advantages: they are cheap, noninvasive, well-tolerated and have a good correlation with MRE [69]. Their use is limited by local expertise and lack of validated score for grading inflammation [70].

Small Bowel Capsule Endoscopy

When there is a strong suspicion for small bowel inflammatory lesions, with normal features at ileocolonoscopy and imaging, small bowel capsule endoscopy (SBCE) represents a valuable and well-tolerated tool. It allows the evaluation of CD small bowel lesions, with no radiation. It is very sensitive for determining mucosal lesions: in comparison to traditional endoscopy, SBCE can evaluate the entire small bowel and is better tolerated from children. Regardless of its many advantages, SBCE also has some weaknesses: biopsy or intervention is not possible, it cannot detect extraluminal processes and there is no way to guide the capsule, so significant lesions may be missed because of a bad orientation of the camera, obscured visualization due to luminal bubbles or debris, or delayed intestinal transit resulting in an inaccurate examination. SBCE is contraindicated in patients with strictures because of the risk of capsule retention. Furthermore, there are no established diagnostic criteria for CD, although most studies have defined the presence of more than three ulcerations in the absence of nonsteroidal anti-inflammatory drug ingestion as a diagnostic criterion, this has not been prospectively validated. Given the concerns about the specificity of SBCE in the diagnosis of IBD, but recognizing its good sensitivity, its utility is greater for monitoring established CD rather than for initial approach [71].

Balloon Enteroscopy

Given the availability of the abovementioned noninvasive tools, the role of enteroscopy, especially balloon-assisted enteroscopy (BAE), remains limited to symptomatic patients in whom ileocolonoscopy, SBCE, and/or cross-sectional imaging are inconclusive for active disease, as well as to obtain mucosal samples to exclude potential malignancies and for therapeutic purposes [72]. Balloon-assisted enteroscopy was first introduced in 2001 as a device offering the possibility of a complete diagnostic and therapeutic access to the entire small bowel with an endoscope [73]. A single-balloon device has also been developed with a similar intention. The enteroscope can be inserted via the oral or anal route and, using the combination of these approaches, a complete examination of the entire small bowel can be achieved in many patients [74]. Several studies support the utility of BAE in established adult and pediatric CD with the potential to affect management in select populations of patients (small bowel disease) [75]. The main limitations of BAE are the invasive nature with the risk of bleeding and perforation (complication rate for diagnostic procedures is around 0.8% but can be as much as 4% for therapeutic interventions), prolonged duration, limited evaluation of the entire small bowel in a one-step approach, and requirement for specialized personnel [75].

Therapy

The traditional management of CD based on achieving prolonged clinical remission has experienced a dramatic evolution in the last years, since the recognized paramount importance of MH, as the main outcome, and, possibly, transmural healing. The so-called treat-to-target (T2T) approach, adapted from rheumatoid arthritis and other chronic diseases, is now a consolidated strategy in the management of IBD: it is focused on an objective measure and monitoring of the intestinal damage at predefined timepoints, and includes therapeutic adjustments in case of failure [76]. The introduction of these targets might be the best way to alter the natural course of IBD by preventing disability and bowel damage [77]. This may be of clinical relevance in children with CD, given the long-term consequences of an early-onset aggressive disease presentation. Pediatric CD therapy employs many of the same treatment regimens as its adult counterpart. There are only a few well-designed clinical trials performed in children, therefore much of the

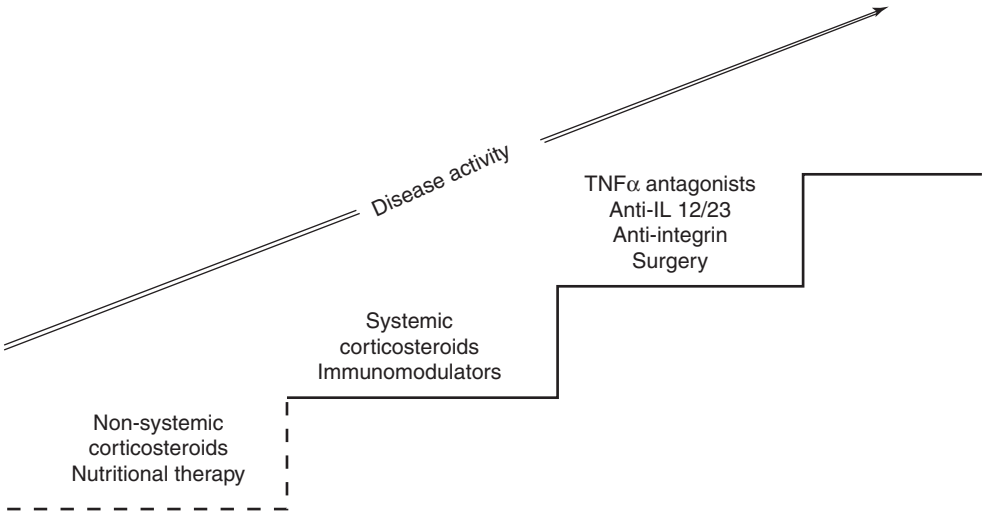

Fig. 28.2 Step-up approach in pediatric Crohn's disease

evidence given is based on adult data. Conventional therapy is based on the escalation of drugs, from those with a better safety profile but a lower efficacy to those with improved efficacy but a greater risk of side effects (steroids, immunomodulators, biologicals, surgery). This "step up" approach is applied in some cases of pediatric IBD. The advantages of "step up" management are mainly to reserve more toxic drugs for those patients with a demonstrable "need" for more intensive therapy [78] (Fig. 28.2). However, potential disadvantages include the observation that conventional therapies have not altered the disease course toward disease complications (strictures and fistulae) or the need for surgical procedures. Hence, an "early aggressive" treatment ("top-down" approach) through the introduction of an early biologic therapy at the diagnosis is now a consolidated treatment strategy for CD patients at high risk of an aggressive disease course [79]. Several risk factors, which prompt this "top-down" treatment, have been identified and include the presence of deep colonic ulcerations on endoscopy, persistent severe disease despite adequate induction therapy, extensive (panenteric) disease, marked growth retardation, severe osteoporosis, stricturing and/or penetrating disease [80]. Increasing the knowledge of these markers (genetic, biochemical, or clinical) of poor prognosis or response to treatment will be the challenge in pediatric IBD research. Figure 28.3 shows the therapeutic pyramid applied in the management of pediatric CD.

Conventional Therapy

Aminosalicylates

There are no randomized controlled trials evaluating aminosalicylate (5-ASA) efficacy for the induction and maintenance of remission in children. Current data in adults do not support their use in ileal CD, showing any (or a slight) improvement compared to placebo, thus their use in CD should not be supported.

Steroids

Conventional corticosteroids (CS) are used for the induction of remission in moderate-to-severe CD. CS are usually quickly weaned after the induction, due to their known adverse effects. Budesonide, an oral steroid preparation that is released in the distal ileum and proximal colon, can be used in patients with mild-to-moderate disease of those segments. It has less systemic side effects than conventional steroids but it is not completely without them, and a quick withdrawal can lead to an adrenal insufficiency [81].

Immunomodulators (Azathioprine, 6-Mercaptopurine, Methotrexate)

Thiopurines, comprising azathioprine (AZA) and its active metabolite, 6-mercaptopurine (6-MP), are widely used maintenance agents in pediatric CD [67]. Their efficacy has been demonstrated in several trials both for induction and maintenance of remission in CD, however, due to their well-known slow onset of action (about 3 months), they are not used for induction of remission. Although the only pediatric prospective, multicenter, double-blind, placebo-controlled trial conducted in children, reported 91% of children receiving thiopurines to be in remission after 18 months of therapy [82], in routine clinical practice, a complete remission can be achieved in about 60% of patients 1 year after beginning the therapy [83]. Myelosuppression is a dose-dependent adverse event that can occur during AZA treatment. Complete blood count and liver tests should be monitored frequently during the therapy. Acute pancreatitis is a rare drug-related adverse event, typically occurring in the first month of treatment [84]. Genetic analysis for thiopurine S-methyltransferase (TPMT) deficiency can help to identify those patients at high risk for hematopoietic toxicity, although normal TPMT

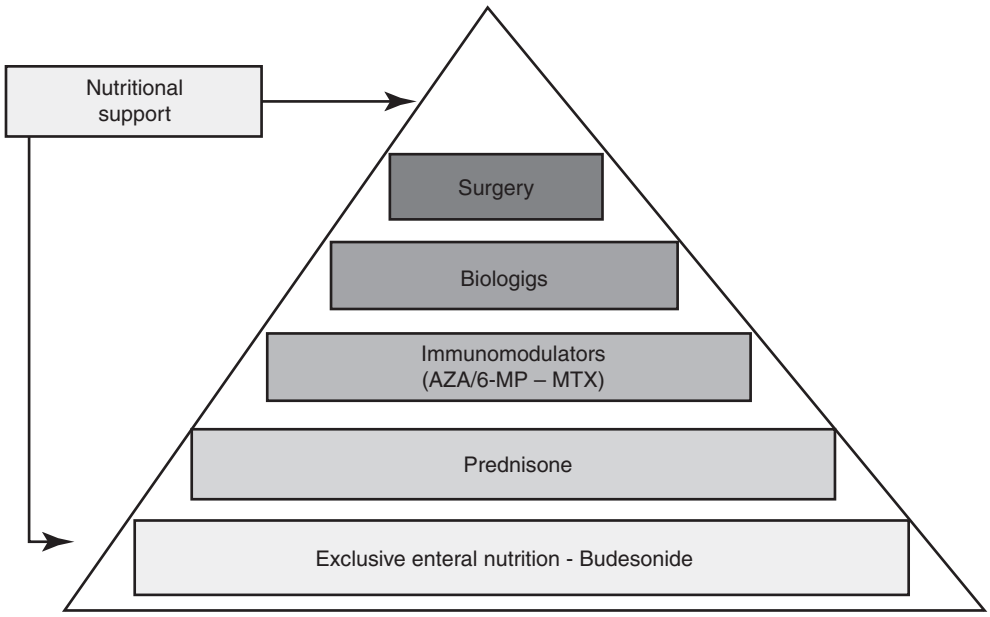

Fig. 28.3 The therapeutic pyramid of pediatric Crohn's disease. (Figure modified from Aloi et al. [78])

testing does not exclude the possibility of leukopenia. Assays for 6-thioguanine (6-TGN) and 6-methylmercaptopurine (6-MMP) levels are commercially available and are related to therapeutic response and hepatotoxicity, respectively. While chronic thiopurine administration has been linked to a potential risk of developing non-Hodgkin (roughly, a four-fold increase in this risk compared to nontreated patients and the general population), AZA may have a role in the risk of developing the hepatosplenic T-cell lymphoma (HSTCL), an aggressive form of non-Hodgkin lymphoma, rapidly evolving and with a poor response and high mortality, mainly occurring in young male patients with IBD, who have received a long-lasting combination of AZA and anti-TNF alpha agents. Methotrexate is often regarded as a second-line immunomodulator in CD patients not responding or intolerant to thiopurines. Some data in adults have demonstrated its efficacy in inducing and maintaining remission [85]. To date, there have been no controlled trials of its use in pediatric CD, but reports from retrospective reviews and uncontrolled trials have shown good remission rates [86, 87].

Biologic Agents

Monoclonal anti-TNF antibodies have revolutionized the treatment and management of IBD since their introduction more than 20 years ago. Infliximab (IFX), the first in this class to be approved in pediatric IBD, is a chimeric monoclonal immunoglobulin G1 (IgG1) antibody (part mouse and part human) that is given intravenously. Infliximab is effective in both inducing and maintaining remission in pediatric CD [88]. It reduces the need for corticosteroid, hospitalization, and surgery, is effective in perianal CD, and induces mucosal healing [89]. In the main pediatric clinical trial, the Realizing Effectiveness Across Continents with Hydroxyurea (REACH) study, children with moderate-to-severe CD received a 3-dose induction of 5 mg/kg IFX at 0, 2, and 6 weeks, followed by 5 mg/kg maintenance infusions every 8 weeks. Remission rates were 60% at week 30 and 56% at week 54 [88]. Moreover, several studies have demonstrated that IFX is able to induce MH [90, 91] and its efficacy seems to be improved when used in combination with AZA, as clearly shown by the Study of Biologic and Immunomodulator Naive Patients in Crohn's Disease (SONIC) trial, in which the combined therapy with IFX and AZA led to a 44% MH rate at week 26, compared to 30% in the IFX group and 17% in those patients treated with AZA monotherapy [59, 92]. In the last decade, therapeutic drug monitoring (TDM), based on the assessment of serum drug concentration and antidrug antibodies, has become an indispensable tool to optimize the use of anti-TNFs. Performed in the setting of active disease ("reactive" TDM) is helpful in elucidating the mechanism underlying the loss of response: an immune-mediated failure (which may benefit a "within-class" switch) is suggested in the presence of high titer of antidrug antibodies with low drug levels, while low drug concentrations in the absence of antibodies indicate nonimmune-mediated pharmacokinetic failure. In this case, treatment optimization through a dose increase or reduced intervals is likely successful. A mechanistic failure, and the need of switching to a non-anti-TNF agent, is implicated in the setting of adequate drug concentrations during active disease [93, 94]. "Proactive" TDM, performed in patients with quiescent disease, to maintain a target trough concen-

tration was recently associated with favorable therapeutic outcomes [95–97]. Whether it should always be preferred to "reactive" TDM is still a matter of debate since the only prospective trials conducted in adults with the aim of evaluating the efficacy of a proactive approach in CD did not confirm its superiority [95, 98].

While it is clear that higher drug levels are associated with better response, IFX optimal serum drug concentration has not been clearly defined. It is suggested that postinduction concentrations greater than 7 μg/mL are more likely to be associated with MH, while during the maintenance phase, through concentrations should not be lower than 3 μg/mL when in remission [93].

Adalimumab is a humanized anti-TNF therapy that has been shown to be effective for induction and maintenance of remission for children with CD [99]. Its efficacy in luminal CD is comparable to that of IFX [100]. Its superiority over placebo in inducing and maintaining remission in patients with moderate-to-severe CD has been largely proven both in adults [101–103] and children [99, 104]. European guidelines on the medical management of pediatric CD recommend its use as an alternative to IFX (according to the availability, route of delivery, patient preference, cost, and local regulations) as the first treatment options for penetrating fistulizing CD, and in children with high risk for poor outcome [67]. TDM applies also to adalimumab: as for IFX, during maintenance therapy, at least 5 μg/mL is recognized as the optimal trough concentration threshold to target, but higher levels (>10 μg/mL) seem to be associated with better therapeutic outcomes [105].

Vedolizumab, an anti-α4β7-integrin humanized immunoglobulin G1 monoclonal antibody and ustekinumab, an anti-interleukin (IL)12/23p40 monoclonal antibody, are emerging as important rescue therapy for patients with moderate-to-severe CD, who fail to respond or lose response to anti-TNF (40% of cases) or, more recently, are biologic naïve. GEMINI 2 and 3 trials of vedolizumab in moderately to severely active CD demonstrated only a partial benefit of vedolizumab on clinical outcomes with approximately 14% of 6-week clinical remission rate [106].

For ustekinumab, the UNITI study reported up to 30% of 6-week clinical remission rate, showing superiority to placebo, with percentage reaching 50% in those patients who were biologic naïve [107].

Nutrition

Exclusive enteral nutrition (EEN) is used as a therapy to achieve remission in children presenting with acute CD [67]. Nutritional therapy consists of using different milk formulas (elemental, semi-elemental, and polymeric) as the primary therapy to induce and maintain remission in CD, as a supplement to improve growth, or to replenish micronutrient deficiency. Although several evidence support the use of enteral nutrition as primary therapy, it has found favor in some parts of the world (mainly Europe and Canada), while it is less widely utilized in others (United States), and can be difficult to administer for long periods of time, mainly because of poor compliance by the patients. Several studies have shown the effectiveness of EEN in achieving clinical remission and mucosal healing in active CD [108–111]. Despite these data, there still remain a multitude of theories explicating the mechanisms of action of EEN. The most frequently advanced theory is that the microbiota of the gut lumen, or more probably products deriving from microbiota, are modified by enteral nutrition [112]; furthermore, the reduction in antigenic load associated with an exclusive enteral nutrition may also contribute to bowel rest [113]. The main drawback of enteral feeding is the poor compliance, most parents are reluctant to commit total enteral nutrition for their children for 6 to 8 weeks as required, and few children are able to consume adequate formula volume by mouth, thus requiring the insertion of a nasogastric tube.

In the last years, new data have emerged about the efficacy of partial enteral nutrition with specific diets for both the induction and maintenance of remission of pediatric CD [114, 115]. Specifically, a few diets from different parts of the world have been evaluated in small prospective and retrospective studies with promising results [115, 116].

Surgery

Up to 30% of children with CD require surgery within 10 years from the diagnosis [117]. The main indications are complications of the disease (especially strictures and fistulae), intestinal perforation or bleeding, failure of medical therapy, and complications of medical therapy (e.g., growth failure). Terminal ileal and colonic disease account for most of surgical interventions in children with CD. Primary goals of surgical treatment in CD are to preserve as much bowel as possible, relieve complications, and to help the patient to achieve the best possible quality of life. In children, the potential for bowel loss due to surgical resections must be weighed against the risk of poorly controlled disease, long-term steroid therapy, and growth failure. The surgical procedure depends on the clinical situation (urgent vs. elective) and the disease extent. Most of the procedures are bowel resections due to strictures, commonly located at the terminal ileum. Stricturoplasty can be an alternative to bowel resection in the case of multiple short segments or longer segments up to 20 cm in length [118, 119].

Another setting of surgical intervention is perianal CD. Several studies have shown that infliximab therapy combined with surgical treatment of fistulizing perineal disease results in significant improvement of perineal disease, which is superior to medical treatment alone [120, 121].

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29 Inflammatory Bowel Disease Unclassified (IBD-U)/Indeterminate Colitis

Barbara S. Kirschner

Introduction

The majority of patients with chronic IBD are diagnosed with either ulcerative colitis (UC) or Crohn's disease (CD) on the basis of established clinical, endoscopic, histologic, and radiologic criteria [1–2]. However, in 5–23% of patients with chronic colitis, a definitive diagnosis of UC or CD cannot be established because the initial macroscopic appearance (either during ileo-colonoscopy or following colectomy) and histologic features overlap between UC and CD [1–9]. In this case, the diagnosis should be IBD-U if made prior to colectomy or indeterminate colitis (IC) if the pathology of the resected colon is not consistent with either UC or CD involving the colon [1–2, 8].

While many of these patients eventually evolve into patterns consistent with UC or CD, approximately 20–60% retain the diagnosis of IBD-U, over 5–10 years or longer post diagnosis [6–13]. This observation is increasingly suggestive that IBD-U represents a separate unique subtype within the spectrum of IBD [1–5, 8]. Updated information describing clinical, histologic, serologic, and newly described genetic features of IBD-U in pediatric patients has been added to this revised chapter.

Inflammatory Bowel Disease Unclassified (IBD-U)

Clinical Presentation

Several studies have estimated the percentage of patients with IBD-U among the patients with chronic IBD (See Table 29.1). A concurrent retrospective/prospective analysis of 1576 children and adolescents from the Italian Pediatric National IBD Register showed that 8% of their study population were diagnosed with IBD-U compared with 52% UC and 40% CD [14]. Presenting symptoms in IBD-U were more similar to UC (bloody diarrhea and abdominal pain) than CD (abdominal pain and diarrhea). Fever and weight loss occurred more frequently in CD than IBD-U and UC: fever in 40.5% CD, 12.9% in IBD-U, and 12.6% in UC; weight loss 50.1% in CD, 17.4% in IBD-U, and 20.6% in UC. The locations within the colon were also similar between IBD-U and UC (pancolitis 34% versus 39%; left colon 27% v. 23%; and rectum only 9% v. 7%). The male to female ratio was lowest in UC (0.82), intermediate in CD (1.18), and highest in IBD-U (1.42). Heyman et al. [15], using the PediIBD Consortium Registry, reported that the prevalence of IBD-U in children with IBD-U was highest in those aged 0–2 years (33%) and decreased to 18% at 3–5 years, 12% at 6–12 years, and 9% in those aged 13–18 [15]. In young chil-

Table 29.1 Proportion of IBD-unclassified (IBD-U) cases in pediatric IBD reported studies

	Total no. IBD Pts	Age group	IBD-U		Mean age at diagnosis (years)		
			No. Pts	(Percent)	IBD-U	v	UC
Everhov [31]	4663	Pediatric	839	18.0%			
Winter [24]	3461		266	7.7%	12.3		12.9
Jose [17]	1649	Pediatric	171	10.3%			–
Chandradevan [5]	136	Pediatric	136	5.8%			
Castro [14]	1576	Pediatric	131	5.3%			
Gupta [7]	420	Pediatric	51	11.9%			
Heikenen [9]	91	Pediatric	9	10.0%	7.8		9.7
Hildebrand [4]	132	Pediatric	36	27.0%	–		
Malaty [32]	420	Pediatric	78	18.6%	9.2		
Meucci [12]	1113	Adult	50	4.6%	–		

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dren (less than 5 years of age), failure to thrive is more prominent than seen in UC [16].

Jose et al. [17] compared IBD disease type with the prevalence of extraintestinal manifestations (EIMs) at the time of diagnosis and during follow-up in pediatric patients with IBD [17]. That study, also from the PediIBD Registry, included 1649 patients:

IBD-U ($n = 171$), UC ($n = 471$), and CD ($n = 1007$). With the exception of primary sclerosing cholangitis (PSC) being more prevalent in UC, the frequencies of the other EIMs did not differ between these disease types.

Epidemiologic Aspects

Sykora et al. [18] noted a broad worldwide variation in the incidence of pediatric IBD with detailed numbers for many countries. For North America, it was highest for CD (0.7–13.9/100,000 person years, 0.5–10.6 for UC, and lowest for IBD-U (0.2–2.1/100,000). A meta-analysis of 14 studies of pediatric patients with IBD and 18 studies in adult patients with IBD showed a higher frequency of IBD-U in children (12.7%) versus adults (6.0%) [19].

Gupta et al. [7] reviewed the medical records of 428 children with IBD followed at the University Chicago. Of those, 49 (11.4%) were diagnosed with IBD-U. Within the IBD-U group, 42.9% had histology "favored ulcerative colitis" (UC) but these patients also had features of CD including areas of focal colitis, focal gastric or duodenal inflammation, anal fissures or isolated granulomas adjacent to ruptured crypts. Features "favoring Crohn's disease" (CD) were present in 20.4% of children with IBD-U, none of whom had granulomas, radiologic evidence of small bowel CD, or perianal findings. In the remaining 36.7%, there were no endoscopic and histologic findings favoring UC or CD. IBD-U was diagnosed after an X-ray study of the upper gastrointestinal tract with small bowel follow-through to exclude the possibility of Crohn's disease.

Heikenen et al. reported a prevalence of IBD-U (10%) in a pediatric population of IBD [9]. As a group, children with IBD-U were diagnosed at a younger age (7.8 years) than those with either UC (9.7 years) or CD (11.4 years).

Criteria for Histologic Diagnosis

In establishing a diagnosis of IBD-U, it is essential to exclude other causes of colitis such as infections (*Clostridium difficile*, Yersinia, *Mycobacterium tuberculosis*, *Entamoeba histolytica*, *E. coli* 0157:B7 or other verocytotoxin-producing strains), drugs (NSAIDS), Behçet's, malignancy, vasculitis, and certain immunologic disorders. Immune deficiency disorders which may include chronic intestinal inflammation are: chronic granulomatous disease, Wiskott-Aldrich syndrome, common variable immunodeficiency disease (CVID), immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX), and glycogen storage disease type 1b [1–2, 8, 20].

Geboes and Van Eyken [8] published an in-depth description of the histologic features observed in normal intestinal mucosa as well as the changes seen in patients with IBD. Focal or diffuse plasmacytosis at the base of the mucosa and crypt architectural changes are strong predictors of IBD. However, it is important to recognize that these findings develop during the course of IBD. Thus, while basal plasmacytosis was seen within 15 days of the onset of IBD, crypt distortion was observed at 16–30 days in only 25% of patients but increased to 75% after 4 months. By comparison with adults, children <10 years of age with new-onset UC show less crypt architectural changes and more rectal sparing than older patients. In addition, 4–8% the initial biopsies were within the normal range. The authors suggested that the presence of basal lympho-plasmacytosis, seen in 58% of cases, should lead to suspicion of underlying IBD. It in this group of children and adults with short history of disease onset, mild basal lympho-plasmacytosis, and no or minimal crypt architecture abnormalities the authors concurred with using the term "inflammatory bowel disease unclassified (IBD-U)."

Patients with IBD-U clearly have IBD but definitive features of UC or CD are absent. IBD-U may also be appropriate for cases of primary sclerosing cholangitis where rectal sparing and patchy or focal inflammation are also more frequently observed [2]. Guindi and Riddell [20] stated that to differentiate indeterminate colitis from Crohn's disease in *resected* specimens, submucosal and subserosal lymphoid aggregates away from areas of ulceration, non-necrotizing granulomas, and skip areas should be absent. This is particularly true where there is nonspecific ileal involvement or gastritis with negative stains for *H. pylori*.

In cases of fulminant colitis, overlapping features between UC and CD (relative rectal sparing, focal inflammation or deep fissuring ulceration) result in a diagnosis of IBD-U in approximately 10–15% of patients [8, 20]. It is also important to recognize that some medications, such as corticosteroids or aminosalicylic acid preparations, may change the diffuse histologic appearance in UC to a more focal appearance [1–2, 8, 20]. Thus, slides from the original or pretreatment colonoscopy should be reviewed when considering a diagnosis of IBD-U [1, 20, 21].

Although histologic criteria would appear to allow differentiation between CD and UC, Farmer et al. (2000) documented disparity among pathologists reviewing cases of colonic IBD [21]. The diagnosis of gastrointestinal pathologists differed from that of the referring institution in 45% of surgical specimens and 54% of biopsy specimens. Of 70

cases initially diagnosed with UC, 30 (43%) were changed to CD or IBD-U or IC, in contrast, 17% of cases initially diagnosed with CD were changed to UC or IBD-U or IC.

The performance of upper gastrointestinal endoscopy with biopsies (EGD) identifies some patients with Crohn's disease whose colonoscopy biopsies are not, by themselves, typical for CD [1–2, 22–24]. McHugh et al. [22] observed that while focal enhancing gastritis (FEG) was highly associated with IBD, 61% in IBD v 11.6 in non-IBD patients, it did not reliably distinguish between UC and CD [22]. Kundhal et al. showed that granulomas in the stomach or duodenum provide evidence confirming the diagnosis of CD even in endoscopically normal-appearing mucosa [23]. Hence, performing an EGD should be part of the initial evaluation of pediatric patients for IBD.

Winter et al. [24] used the EUROKIDS Registry to assess the frequency of change in diagnosis from IBD-U in children provisionally diagnosed with IBD-U. After undergoing repeat endoscopic and/or radiologic re-evaluations, mean 5.7 years after the IBD-U diagnosis, 20% children were diagnosed with UC and 10% CD. The authors noted that an initial incomplete diagnostic workup likely contributed to this change in diagnosis, since only 73% of the CD group had undergone small bowel imaging. They emphasized the importance of endoscopic or small bowel imaging in patients with active disease attributed to IBD-U.

Serologic Markers and Defining IBD Categories

Gupta et al. [7] evaluated the role of p-ANCA and anti-*Saccharomyces cerevesiae* (ASCA) to determine whether serology could identify UC and CD in pediatric patients previously diagnosed as IBD-U [7]. While p-ANCA was positive in 68% of those favoring UC, and ASCA positive in 37% of those favoring CD, 86% of patients with IBD-U were negative to both p-ANCA and ASCA.

Joossens et al. [10] correlated serologic markers in 97 adult patients initially diagnosed with IBD-U. After a mean follow-up of 6 years, 32% of adult patients with IBD-U were reclassified as having UC or CD, half of whom were positive for p-ANCA or ASCA. However, almost half of the patients with IBD-U (48.5%) remained p-ANCA/ASCA negative and continued with that diagnosis 10 years after the initial diagnosis.

Birimberg-Schwartz et al. [25] analyzed IBD serology patterns in 146 pediatric patients with IBD-U followed in the IBD Porto Group of ESPGHAN. As reported in adults, the most common pattern was p-ANCA−/ASCA− in 41% but other patterns were present as well including p-ANCA+/ASCA− in 34% and p-ANCA−/ASCA+ in 17%. The authors concluded that IBD serology was a poor discriminator to identify patients with IBD-U.

Radiologic Imaging

The most frequently used diagnostic studies (after ileocolonoscopy and EGD) for distinguishing among the various forms of pediatric IBD are those which image the small intestine such as magnetic resonance enterography (MRE) or small bowel follow-through (SBFT). Because of the ionizing radiation associated with SBFT, utilization of MRE is favored in children with IBD-U, UC, and CD [26]. MRE in combination with oral contrast, used to distend the small bowel, demonstrates increased wall thickness in children with CD while those with IBD-U and UC showed mild parietal contrast enhancement but not bowel wall thickening.

Capsule Endoscopy (CE)

Small intestinal capsule endoscopy (SBCE) has the potential to identify lesions in the upper GI tract, jejunum, and ileum which would allow differentiation between IBD-U and CD after conventional diagnostic modalities. Gralnek et al. [27] used SBCE to determine whether ulcers could be observed in four pediatric patients previously diagnosed UC or IBD-U, who were experiencing disease exacerbation. Based on abnormal mucosal findings, two of the four patients were reclassified with CD. In addition, 8 of 10 patients with "suspected IBD" were also found to have ulcerations and diagnosed with CD.

Goldstein et al. [28] performed SBCE in adult postcolectomy patients with IC (n = 20) and UC (n = 49) and were unable to identify patients who would be reclassified as de novo CD following IPAA. These authors cautioned that since SBCE may identify lesions in healthy individuals, as well as those who take NSAIDS, the specificity of mucosal these lesions is unclear.

Kopylov and Seidman [29] reported that the most common cause of lesions which mimic pediatric small bowel CD is NSAID medication. The authors noted that the lesions were indistinguishable from CD, occur as early as two weeks of use, and may be seen in up to 70% of chronic NSAID users. They advised instructing patients who will undergo CE to avoid NSAIDs for at least one month before the examination.

Another confounding variable is that small bowel inflammation was observed in a significant number of patients with established UC compared with non-IBD patients [30]. Of 23 UC patients, 13 (57%) showed small-bowel lesions and 8 (35%) had erosions, as opposed to 2/23 (7%) and 1/23 (4%)

in the control group. These findings emphasize the potential risk of misdiagnosis in IBD patients.

Natural History

With time, 50–72% of adult patients and up to 64% of pediatric patients with IBD-U are reclassified as definite UC or CD during subsequent observation [1–6, 14, 19, 25]. Moum et al. observed that of 36 patients initially diagnosed as IBD-U, 33% were reclassified as UC and 17% as CD after a follow-up period averaging 14 months [11]. Meucci et al. [12] reported that 37/50 patients (72.5%) changed from IBD-U to a definite diagnosis of UC or CD during follow-up with a cumulative probability of 80% within 8 years of diagnosis [12]. In contrast, Wells et al. followed 16 patients with IBD-U for a mean of 10 years and observed 3 were reclassified with UC, 1 with CD but 75% remained IBD-U [13].

The course of IBD-U in 36 Swedish children after a mean follow-up of 4.6 years was analyzed by Hildebrand et al. [6] Their findings were similar to those as described above in adults: 21/36 (58%) were subsequently categorized as UC, 2/36 (6%) CD, and 13/36 (36%) remained as IBD-U. Everhov et al. [31] in a later study of Swedish children reported that they were more likely to change their IBD subtype than adults over 3.8 years (29% v 17%): CD 43–44%, UC decreased 45–38%, and IBD-U increased from 12% to 18%.

In our series of 49 children with IBD-U, nine had undergone colectomy at a mean of 24 months after diagnosis of IBD-U [7]. After a mean follow-up of 42 months, 6/9 with postsurgical diagnosis of IC had repeat endoscopic examinations resulting in reclassification consistent with UC in 3/9 (33%), IC in 2/9 (22%), and CD in 1/9 (11%). Malaty et al. [32] described a 12-year telephone follow-up of 20/60 pediatric patients who had been previously diagnosed with IBD-U The range of outcomes was broad: 30% remained IBD-U, 10% reclassified as CD, 5% as eosinophilic colitis but 55% reported a complete resolution of their symptoms.

Medical Therapy

The observation that many patients with IBD-U are reclassified as UC or CD has made it difficult to establish whether IBD-U represents a separate form of IBD. Perhaps because of the smaller number of patients with IBD-U and the variability of their histologic findings, the response to various drug regimens in this population has not been specifically addressed.

In our program, the choice of therapeutic intervention (see Table 29.2) is selected depending on the severity of symptoms, extent, and severity of endoscopic and histologic findings and laboratory parameters. Given the current state of knowledge of IBD-U, most pediatric patients receive drug therapy which is similar to that indicated for UC with comparable extent and severity. These include 5'aminosalicylic acid (5'ASA, mesalamine) preparations for mild disease and corticosteroids and immunomodulatory and/or biologic therapy for moderate and severe disease. In the past, immunomodulatory agents, such as azathioprine or 6-mercaptopurine, were used in approximately 60% of our pediatric population with IBD due to the presence of steroid dependency, resistance, or toxicity. More recently, there has been a trend among pediatric gastroenterologists to use methotrexate for immunomodulation when concomitant therapy is indicated. The expectation is that there may be a lower risk of malignancy, especially hepatosplenal T-cell lymphoma, than with the combination of thiopurine and anti-TNF therapy.

Table 29.2. A medical approach to the pediatric patients with IBD-U

5'Aminosalicylic acid preparations (5'ASA, mesalamine) for mild disease
Corticosteroids (moderate to severe disease severity)
Immunomodulators, if indicated
Azathioprine (AZA) or 6-mercaptopurine (6-MP)
[Measure thiopurine methyltransferase (TPMT) prior to use to exclude deficiency
Monitor RBC 6-thioguanine (6-TG) levels, CBC, ALT to reduce risk of toxicity
Methotrexate for combined therapy
Biologics: Anti-TNFα preparations for moderate to severe disease.
No published studies for anti-α4β7 integrin, anti- IL-12/23 in pediatric IBD-U.

*Note: There are no published pediatric trials which have specifically addressed the response to these medications in patients with IBD-U. These recommendations are based on the limited clinical reports of use in pediatric patients with IBD-U [4–5] and published guidelines for ulcerative colitis in pediatric patients

Some clinical trials of adult patients with IBD have included small numbers of patients with IBD-U although not specifically addressing therapeutic response in this group. Clinical response to infliximab in 20 adult patients with refractory IBD-U was reported [33]. In that study, 14/20 (70%) showed a "complete clinical response" and 2/20 (10%) "partial response."

In the absence of clinical trials in pediatric IBD-U patients, Aloi et al. [4] reviewed various treatments and outcomes of 797 pediatric patients with isolated colonic IBD and compared results between groups of patients with IBD-U, UC, and CD after mean follow-up of 2.8 years. Patients with IBD-U were more likely to have received 5-ASA than those with UC (88% v 71%) and less likely to be treated with corticosteroids than UC (59% v 71%) or immunomodulators than CD (26% v 52%). At follow-up, remission or mild disease (PGA < 1) in IBD-U was similar to UC (69% v

64%) but significantly more frequent than CD (46%, $P = 0.0001$). Biologics were also similar between IBD-U and UC (17%, 14%) but used less often than CD (34%, $P = <0.001$). The authors concluded that IBD-U tends to have a milder course than UC and CD with less requirement for corticosteroids, immunomodulators, and biologics and suggested that mesalamine could be considered for the initial therapy of pediatric patients with IBD-U. Favorable outcomes in pediatric patients with UC and CD suggest that anti-TNF agents will also be effective in some patients with IBD-U who are not responding to conventional medications.

Surgical Treatment

Conflicting data has been published regarding medical failure and complication rates following medical and surgical intervention in patients with IBD-U versus UC. Some centers report that IBD-U is more refractory to medical intervention than UC resulting in a greater relapse rate and subsequent need for colectomy. In contrast, Witte et al. [34] described similar response rates to medical intervention in IBD-U and UC for patients enrolled in the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). "Complete relief of complaints" was noted in 48% of UC patients versus 50% of those with IBD-U. Partial response was also similar in both groups: 37% of patients with UC "improved" versus 33% with IBD-U.

One of the most important reasons for distinguishing IBD-U from UC and CD is for counseling the patient and family about the potential increased risk for complications and possible subsequent change in diagnosis following IPAA. Higher rates of pelvic sepsis, fistula formation, and pouch failure after ileal pouch-anal anastomosis (IPAA) occur in patients diagnosed with IC after surgery versus UC [35]. A potential positive finding is that patients diagnosed with IC after surgery who are p-ANCA and ASCA negative have lower risk for pouchitis, pouch complications, and the development of Crohn's disease [36]. Thus, obtaining these serologic tests in patients being evaluated for IPAA may provide useful predictive information [37].

It is our practice to repeat colonoscopy, usually with concurrent EGD, during selected periods of relapse, if a higher level of therapy is being considered to assess whether histologic changes consistent with UC or CD have developed in patients with IBD-U. This is especially the case if colectomy and ileal pouch anal anastomosis (IPAA) are being advised because of refractory disease. Part of the reassessment should include repeat small bowel x-ray imaging so that patients with ileal CD would be excluded from IPAA. Patients with persistent IBD-U are counseled regarding the potentially greater risk of pouch complications. Patients diagnosed with IC after reviewing the colon pathology undergo a multistaged operative course consisting of a total abdominal colectomy with temporary ileostomy and Hartmann pouch. In this way, the entire resected colon can be assessed to exclude Crohn's disease prior to creating the IPAA.

Multicenter Collaborative Studies Supporting IBD-U as a Separate IBD Subtype

Two recent large multicenter pediatric studies have further advanced the concept of IBD-U as a separate and intermediate subtype between Crohn's disease and typical ulcerative colitis. Birimberg-Schwartz et al. [38] using the Porto Group Registry published criteria with a goal of standardizing the classification of the different subtypes of IBD (small bowel CD, isolated Crohn's colitis, IBD-U, atypical UC, and typical UC), but focusing on those with the isolated colon phenotype [38]. The authors proposed a list of 23 features based on the presence or absence of perianal findings, macroscopic appearance on endoscopy, histology of mucosal biopsies, and abnormal radiologic findings such as thickened bowel loops or fistulae which were placed into one of three classes. Class 1 contained features consistent with Crohn's disease and these patients were excluded from further analysis. Class 2 also had features suggestive of Crohn's, such as rectal sparing, ileitis which could be backlash, positive ASCA with negative p-ANCA, but which could occur in up to 5% of UC cases. Class 3 included focal colitis on histology and <5 aphthous ulcers in the stomach or colon which can occur in 5–10% of UC cases.

At follow-up, mean 2.8 years, 79% of pediatric patients diagnosed as IBD-U maintained their diagnosis and 21% changed to UC or CD. The major feature associated with a change in diagnosis to CD was the presence of ileitis with only mild inflammation in the ascending colon. This is in contrast to the backwash ileitis seen in ulcerative colitis win association with severe pancolitis with cecal involvement.

Macroscopic upper gastrointestinal involvement on endoscopy was statistically different between the three groups with IBD-U being intermediate between CD and UC: CD 53%, IBD-U 41%, and 28% in UC. Although the follow-up was of short duration, the authors concluded that their low reclassification rate supported the concept that IBD-U is a "true intermediate phenotype." IBD serology did not improve the accuracy of the algorithm.

Recently, Dhaliwal et al. [39] compared the clinical IBD diagnosis with that predicted using the PIBD classes algorithm in a group of 62 pediatric patients with colonic IBD (UC, CD, or IBD-U) prior to colectomy with the postcolectomy histologic diagnosis. Clinical diagnosis was more con-

cordant with the postcolectomy diagnosis in 58/62 patients (94%) compared with the IBD classes algorithm concordance in 51/62 patients (82%).

In 2018, Chandradevan et al., using data from the RISK study population, compared IBD serology and RNASeq gene expression profiles in terminal ileal and rectal biopsies from an inception cohort of pediatric patients with IBD-U, CD, and UC [5]. In this population of 1411 subjects, 136 were initially diagnosed with IBD-U. After 2 years of follow-up, 60% of subjects remained IBD-U, 26% were reclassified as UC, and 14% as CD. Both serology profiles and mucosal gene expression profiles in the IBD-U group were very similar to UC and dissimilar to CD. With regard to serology, the only difference between the IBD-U and UC groups was a higher percentage of ANCA+ in the UC group (64.9% v 43.5%). In contrast, three serologies (ASCA, CBir1, and OmpC) were statistically different between CD and IBD-U ($P < 0.0001$).

Gene expression profiles between IBD-U and UC in both ileal and rectal biopsies were similar with a small number of differences between them 15/5084 (TI) and 26/16,408 (rectum). In contrast, differences in gene expression profiles were much greater between IBD-U and CD, 5084/5094 (TI) and 2645/16,408 (rectum). These results suggest that IBD-U is more like UC than CD on a molecular basis. Clinically, anti-TNFα was required by significantly more patients with CD (50.3%) compared with UC (27.8%) and IBD-U (24.2), $P = <0.0001$. In this short follow-up period, no disease-modifying effects were noted in patients with IBD-U or UC who received anti-TNFα compared with reduction in penetrating disease observed in the RISK CD population which was published in 2017 [40].

Conclusions

IBD-U is being increasingly recognized as a separate subtype in the classification of chronic IBD, remaining separate from UC and CD in many cases after long-term follow-up. In young patients or those with recent-onset symptoms, the histologic features are less likely to have developed typical features [1, 8]. Patients and families should understand that IBD-U is not indefinite colitis but rather a form of chronic inflammatory bowel disease which may either remain as IBD-U or become more consistent with UC or CD over time. The clinical observations from many groups, as described above, conclude that the course of IBD-U is more like UC than CD and therapies favored for UC should be considered in the treatment strategies for IBD-U. The results emanating of the RISK study by Chandradevan et al. provide mucosal gene expression profile and IBD serology associations to support IBD-U as a separate form of IBD [5].

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Ulcerative Colitis

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Anita Rao and Ranjana Gokhale

Introduction

Chronic inflammatory bowel diseases (IBD), which include Crohn's disease (CD), inflammatory bowel disease unclassified (IBDU) and ulcerative colitis (UC), are important causes of gastrointestinal disease in children and adolescents. The early age of disease onset in some children, variable clinical presentations, therapeutic challenges, as well as emotional needs of children and their parents pose difficult challenges to the gastroenterologist.

Epidemiology

UC has an increasing incidence and prevalence worldwide, especially in adolescents and young adults. The incidence of UC may vary from 0.5 to 31.5 per 100,000 people each year, depending on the studied population. In general, the disease has a high occurrence in Western countries such as those in North America and Northern/Western Europe compared to Asian countries. However, incidence rates have plateaued or even decreased in Western countries over time, and in contrast incidence has been steadily increasing in more developed countries in Asia as well as in Central and South America. In Asia in particular, the incidence of IBD is 1.4 cases per 100,000 people and climbing [1].

There is also a geographical gradient for the incidence of IBD, with higher rates in the north as compared to the south [2]. Additionally, pediatric-onset IBD continues to increase steadily. As IBD is a chronic disease with relatively low mortality that is diagnosed primarily in young people, the prevalence of IBD increases over time such that new diagnoses add to the base population at a rate significantly higher than the loss of patients from a clinical practice [1].

According to a single-center prospective study by Capone et al. there was an increase in the prevalence of IBD for first-degree relatives and all relatives at 20 years from the time of diagnosis by 12.9% and 13.7%, respectively. Additionally, positive family history at diagnosis was associated with a 2-fold greater likelihood of subsequent positive family history at 20 years. This suggests an increased role of environmental factors and lifestyle effects on the pathogenesis of UC [3].

The female to male ratio for UC differs between 0.51 and 1.58, indicating that UC is not sex specific. Any age group from infants to the elderly can be affected, but the peak age of onset is between 15 and 30 years with a second but smaller peak between 50 and 70 years. About 20 to 30% of patients with UC and CD have the onset of their symptoms below the age of 18 years, and about 5% of cases occur before age 10 years [4].

Pathogenesis

UC can be considered an immune-mediated disorder that develops in genetically predisposed individuals because of dysregulated immune responses against environmental and intraluminal antigens [5]. More recently the focus of research has shifted to examining the interplay between the environment (mainly the intestinal flora) and the defense mechanisms of the intestinal barrier (mainly the mucosal layer and the mucosal immune system). These aberrant immune responses to commensal microbes likely result in lesions of the intestinal mucosal layer involving extensive epithelial damage, immune infiltration, crypt abscesses, and chronic inflammation, which are hallmarks of UC [6].

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Genetics

In a recent meta-analysis of genome-wide association studies (GWAS) for CD and UC, more than 200 IBD-associated loci were identified. Many of them are associated with both UC and CD. These regions contain candidate genes for a variety of functions like autophagy, microbe recognition, lymphocyte signaling, response to endoplasmic reticulum stress, and cytokine signaling, among others. While GWAS has uncovered many new pathogenesis pathways, each locus only has a very small to modest effect size, with the exception of a strong association for the human leukocyte antigen (HLA) loci on chromosome 6 that has consistently shown a large effect on UC susceptibility [7].

UC is more common in the Jewish population. The lower concordance rate in monozygotic twins of 15% and in dizygotic twins of 5% in UC, compared with 50% in monozygotic twins and 20% in dizygotic twins in CD, indicates that genetic contribution in UC is weaker than in CD [8]. In the case of "very early onset" or VEO-IBD, mutations of the genes *IL-10RA*, *MSH5*, and *CD19* are associated with disease development [9].

Environmental Factors

Increasing incidence particularly in industrialized countries indicates environmental influences in the development of UC. In fact, cesarean delivery, lack of exposure to breast milk [10], increased dietary fat intake (i.e. a "western diet"), and early exposure to antibiotics have all been implicated as risk factors for IBD. Patients with newly diagnosed UC are more likely than age-matched controls to have a history of gastroenteritis [11]. Interestingly, early appendectomy prior to the age of 20 years is associated with decreased risk of UC [12]. In a meta-analysis of 13 studies examining the relationship between UC and smoking, there was an association between former smoking and the development of UC, with current smoking having a protective effect on the development of UC compared to controls [13, 14].

Children of persons who emigrated from an area of low prevalence to one of high prevalence show an increased risk of UC than the immigrants themselves, suggesting that environmental factors in early life that affect the developing immune system and microbiome are essential to UC pathogenesis [5, 15]. The natural geographic distribution of IBD, with higher rates seen in the north and lower rates seen in the south, introduces the question of whether vitamin D and sunlight are protective factors.

UC and the Microbiome

The IBD gut is characterized by reduced microbiome diversity and a depletion of protective bacteria such as short-chain fatty acid (SCFA)-producing Ruminococcaceae and Lachnospiracaea that coincides with an expansion of pro-inflammatory microbes such as Enterobacteriaceae, including *Escherichia coli* and Fusobacteriaceae [6]. SCFAs including butyrate are a major fuel source for colonic epithelium and are associated with better gut health; notably, oxidation of butyrate is impaired in UC patients [16]. Blooms of *Ruminococcus gnavus* strains and an increase in facultative anaerobes co-occur with more severe IBD activity. Microbes also show protective effects in mouse models, and *Bacteroides fragilis* mono-colonization has been shown to protect against induced colitis. Depletion of butyrate-producing *Faecalibacterium prausnitzii* in IBD has been reported previously in the literature, and animal studies have shown that *F. prausnitzii* inhibits IL-17 and suppresses Th17 activity, suggesting an association between this bacterium and reduced mucosal inflammation. Topical butyrate is effective as an adjunct therapy for UC, and butyrate enemas have been shown to reduce mucosal inflammation in distal UC, indicating a benefit of increased butyrate levels. The anti-inflammatory activity of butyrate in UC has been associated with inhibition of NF-kB activation in lamina propria macrophages, reducing cytokine secretion and inflammation [6]. Early onset dysbiosis may cause immune disruptions that result in microbiome intolerance by the host. Factors that cause early-life dysbiosis, including antibiotic usage, have gained more attention as the incidence of pediatric IBD has increased dramatically [17].

Clinical Manifestations

UC always affects the rectum and extends proximally in a symmetric fashion involving variable lengths of the colon. Involvement could be limited to the rectum (proctitis), or can extend to the distal colon (up to splenic flexure or left-sided colitis) or the entire colon (pancolitis). Children tend to have a higher likelihood of pancolitis at presentation as well as proximal extension of disease over time compared to adults [18]. In fact, 60–90% of pediatric UC presents with pancolitis, which is twice as often as adult UC. Additionally, pediatric UC carries a 30–40% colectomy rate at 10 years, compared to 20% in adult UC. Childhood-onset disease is also characterized by a higher risk of corticosteroid dependency and earlier immunosuppressive therapy introduction [19].

Intestinal Manifestations UC is usually diagnosed earlier after the onset of symptoms than CD, as rectal involvement leads to the presence of gross blood in the stools alerting parents and physicians to a gastrointestinal problem. The most consistent feature of UC is the presence of blood and mucus mixed with stool, accompanied with lower abdominal cramping that is most intense during the passage of bowel movements. Patients may also experience tenesmus, which a sensation of needing to evacuate stool. The location of abdominal pain depends on the extent of colonic involvement. Pain in the left lower quadrant is associated with distal disease and extends to the entire abdomen with pancolitis. Systemic symptoms including fever, anorexia, weight loss, and anemia may occur with fulminant colitis [11].

Nevertheless, the diagnosis of pediatric-onset UC may be more challenging due to the existence of atypical phenotypes. "Atypical UC" is suggested when features not characteristic of classic UC are present, but common enough in UC to preclude the diagnosis of CD. In particular, six different atypical phenotypes have been recently identified in the revised criteria from the Pediatric IBD Porto Group of the European Society of Pediatric Gastroenterology, Hepatology, & Nutrition (ESPGHAN) for the diagnosis of pediatric UC: rectal sparing, short segment disease, cecal patch, upper gastrointestinal (UGI) findings, acute severe colitis (ASC), and "backwash ileitis" [19]. The presence of ileitis may complicate the potential diagnosis of UC, and although UC is restricted to the colon by definition, nonspecific mucosal inflammation in the terminal ileum (called "backwash ileitis") may be found in up to 20% of UC patients [20]. According to the Porto criteria, backwash ileitis should entail a "short segment of nonstenotic erythema or edema in the presence of pancolitis including the ileocecal valve, without granulomata or deep ulcers" which would suggest CD [21].

Extraintestinal Manifestations

As IBD is a systemic disease that can involve multiple organs, patients with IBD often exhibit extraintestinal manifestations (EIM). In up to 28% of pediatric patients, EIMs are present at diagnosis [18]. In fact, rates of EIM at IBD onset are higher in children compared to adults [22]. EIMs are more commonly seen in CD compared to UC (30–71% vs. 21–22%). EIM's are usually related to disease activity but may precede, develop concurrently, or may also occur after a colectomy [18]. Data from the PediIBD Consortium Registry including 1649 children with IBD show a cumulative incidence of EIMs of 9% at 1 year, 19% at 5 years, and 29% at 15 years after diagnosis. Thus, in 29% of pediatric IBD patients who do not have EIMs at the time of diagnosis, at least one EIM will develop within 15 years [23]. Since up to 35% of pediatric IBD patients may manifest EIM prior to intestinal symptoms, IBD should be suspected in children with EIMs to prevent delays in diagnosis and treatment [18]. EIMs predominantly involve four organ systems: skin, joints, biliary tract, and eyes. Skin-related EIMs include erythema nodosum, pyoderma gangrenosum, psoriasis, and aphthous stomatitis. Eye-related EIMs include episcleritis and uveitis. Other EIMs include peripheral arthritis, axial arthropathy, osteoporosis, primary sclerosing cholangitis, and chronic active hepatitis [5].

Arthralgia and Arthritis

Arthralgia and arthritis occur frequently in about 5–20% of children with UC and may occasionally precede intestinal manifestations of IBD [24]. They usually coincide with disease activity and improve with medical treatment of underlying intestinal inflammation. They can be classified into two forms: peripheral arthropathy and axial arthropathy (AS) [25]. Peripheral arthropathy or arthralgia is usually pauciarticular affecting large joints, such as knees, ankles, hips, wrists, and elbows in decreasing order of frequency. Joint deformities are usually not seen.

Ankylosing spondylitis (AS) is more common with UC and is associated with HLA B27 in 50 to 80% of cases. Progression is variable and does not appear to correlate with severity of bowel symptoms. Colectomy does not affect the course of AS. Sacroileitis is usually asymptomatic and may be detected on bone scans.

Chronic recurrent multifocal osteomyelitis (aseptic inflammation of the long bones and clavicles) and hypertrophic osteoarthropathy (digital clubbing, painful swelling of limbs, arthralgia, joint effusions) are uncommon but have been described. Both of these conditions are managed by treating the underlying colitis [26].

Mucocutaneous Lesions

Oral aphthous ulcers occur less commonly with UC (5%) as compared to CD. Ulcers usually cause minimal discomfort although they may occasionally cause debilitating pain. They tend to parallel disease activity and treatment is directed towards the underlying disease.

Pyoderma gangrenosum (PG) is a deep severe ulceration of the skin and is an unusual manifestation with UC (<1%). Lesions can be multiple in number and are typically located below the knees. Biopsy of the lesion shows neutrophilic infiltration with abscess surrounded by a lymphocytic vasculitis. PG usually parallels active colonic disease but on occasion may be refractory to treatment and may require intensive

local therapy, corticosteroids (CS), minocycline, dapsone, clofazimine, cyclosporine, or infliximab.

Erythema nodosum is characterized by the development of painful, indurated, purplish red, ovoid nodules 1 to 3 cm in diameter, most commonly seen over extensor surfaces. Erythema nodosum is less common in UC, and improvement coincides with the treatment of the bowel disease [27].

Ophthalmologic Disease

Eye abnormalities are described in approximately 1.6 to 4.6% of children with UC [28]. Iritis and uveitis are associated with the presence of the human leukocyte antigen HLA-B27 and typically run a course independent of the bowel disease. They present with eye pain, headache, and blurred vision or may be asymptomatic and detected by slit lamp examination. Treatment consists of pupillary dilatation, covering the eye to decrease pain and photophobia and local or systemic corticosteroids. Episcleritis usually is related to disease activity and presents with scleral and conjunctival erythema with a burning sensation and photophobia. Local corticosteroid drops are usually effective.

Hepatobiliary Disease

Primary sclerosing cholangitis (PSC) is usually seen in association with UC. PSC is a chronic progressive cholestatic disease characterized by inflammation and fibrosis of the intrahepatic and extrahepatic biliary tree resulting in multifocal strictures. It is diagnosed based on a cholestatic biochemical pattern and a characteristic "beaded" appearance on cholangiography, which defines large duct, or "classic" PSC. In contrast, "small duct" PSC is only identifiable by liver biopsy without the presence of cholangiographic abnormalities. It is a less common variant with a more benign course. PSC may be asymptomatic and is detected because of elevated alkaline phosphatase and γ-glutamyltransferase (GGT) during routine blood screening. Patients may occasionally present with pruritis and PSC prior to the development of UC. The course of PSC appears to be unrelated to underlying bowel disease and may progress even after a colectomy. Peripheral antineutrophilic cytoplasmic antibodies (pANCA) are positive in most patients with PSC and may be a marker for genetic susceptibility for this disease.

Colorectal cancer (CRC) has been associated with concomitant PSC and IBD, with patients having a higher risk of CRC at a younger age when compared to non-IBD PSC patients [29, 30].

Autoimmune hepatitis (AIH) is also seen in association with UC. Diagnosis is made following a liver biopsy, which shows lobular inflammation with piecemeal necrosis. Treatment includes CS and immunosuppressive medications. As with PSC the course of AIH can be independent of UC.

Diagnostic Criteria

The diagnosis of UC is based on clinical presentation and then confirmed by laboratory screening tests, radiologic examination, endoscopic appearance, histopathology, and serological findings. Additionally, it is extremely important to exclude the presence of enteric pathogens before confirming the diagnosis of UC. Lennard-Jones suggested the following criteria for the diagnosis of UC: contiguous mucosal inflammation without granulomata, always involving the rectum and extending continuously in various degrees to a part or the whole colon [31]. However, universally accepted well-defined criteria or a point score for classification of UC do not exist. Abnormalities like complex or fistulizing anal lesions, involvement of the upper gastrointestinal (UGI) tract, skip lesions, or granulomata are highly suggestive of CD [5].

Endoscopic and Histological Features

All patients should undergo endoscopic evaluation (colonoscopy and esophagogastroduodenoscopy) with biopsies taken from all segments of the intestine as part of medical workup. Endoscopic features of UC include continuous mucosal ulcerations starting from the rectum with erythema, friability, and loss of typical mucosal vascular pattern. The Ulcerative Colitis Endoscopic Index of Severity (UCEIS) is a method in which to grade endoscopic severity of disease that includes three descriptors: vascular pattern, bleeding, and erosions or ulcerations. The final UCEIS score ranges from 0 to 8, with higher scores denoting increasing disease activity (See Fig. 30.3) [32].

Histological, UC is characterized by diffuse inflammatory cell infiltration of the mucosa with basal plasmacytosis, crypt architectural distortion, cryptitis/crypt abscesses, and a reduction of mucus-secreting goblet cells (See Figs. 30.1 and 30.2) [5, 33] (Figs. 30.3 and 30.4).

Laboratory and Serological Markers

Laboratory findings are important screening tests in the diagnosis and monitoring of UC; however, there are no disease-specific markers yet identified. Common laboratory findings include an elevated white blood cell count, thrombocytosis, elevated inflammatory markers (CRP, ESR, and fecal calprotectin), and measures of deficiencies due to

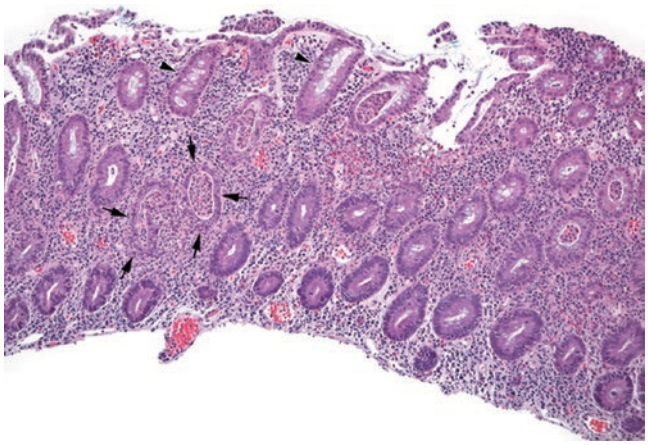

Fig. 30.1 Representative acute inflammatory features. In contrast to relatively well-preserved crypts (arrowheads), crypt abscesses (arrows) are dilated, lined by attenuated damaged epithelium, and contain acute inflammatory cells in the epithelium and the lumen of the crypt (hematoxylin and eosin). (Reprinted with permission from Boyle et al., "Histologic Correlates of Clinical and Endoscopic Severity in Children Newly Diagnosed With Ulcerative Colitis")

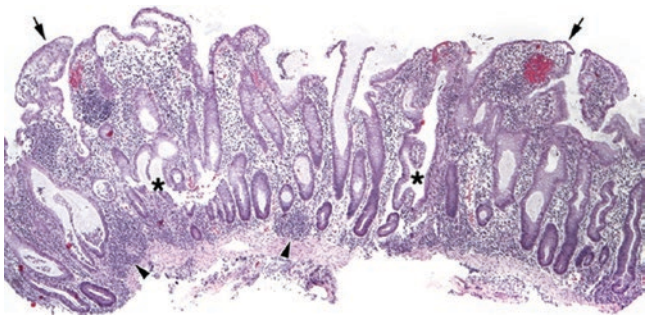

Fig. 30.2 Representative architectural and nonarchitectural features. Surface villiform changes are evident (arrows), elongated crypts are easily identified, crypts with abnormal shapes are seen (asterisk), as are subcryptal lymphoid aggregates (arrowheads) (hematoxylin and eosin). (Reprinted with permission from Boyle et al., "Histologic Correlates of Clinical and Endoscopic Severity in Children Newly Diagnosed With Ulcerative Colitis")

increased losses from diarrhea (iron deficiency anemia, hypoalbuminemia) [34].

On presentation, serum inflammatory markers (CRP, ESR) are usually higher in CD compared to UC. In a cohort of 512 children with the new diagnosis of UC, 54% of those with mild disease had normal results on four common laboratory assays (hemoglobin, albumin, platelet count, and ESR), compared with 21% of children with mild CD [35]. ESR and CRP are fairly correlated with colonic inflammation, with a slight superiority of CRP [34]. Fecal calprotectin levels above the cutoff of 100 μg/g have been shown to correlate with mucosal inflammation in UC and are considered to be superior to inflammatory markers in the blood, which may be nonspecific. Fecal calprotectin is also useful for long-term follow-up and in differentiating IBD from functional causes [34].

Generally, serological antibodies related to IBD encompass either autoantibodies or antibodies targeting microbial antigens. Particular microbial antibodies include antibodies directed against the yeast *Saccharomyces cerevisiae* (ASCA), *Escherichia coli* outer membrane porin C (Omp-C), and flagellin (cBir1), which suggests that commensal flora may be triggering a deleterious immune response in patients with IBD [36].

The most frequently studied serological markers in IBD are perinuclear antineutrophil-cytoplasmic antibodies (pANCA) and antibodies against *Saccharomyces cerevisiae* (ASCA). pANCA can be found in 50–70% of UC patients and in less than 10% of CD patients, mainly in those that have a "UC-like" phenotype. pANCA positivity and a negative test for CD-specific ASCA indicate that UC is more likely than CD. In patients with IBD-U, the results of both pANCA and ASCA can aid in making a definitive diagnosis [37].

Previous studies have shown that pANCA titers can be used to stratify patients into distinct subgroups. In a pediatric study from 1998, Ruemmele et al. observed differences in pANCA titers within subgroups of IBD, with equally high titers in both UC-like CD with pancolitis (median of 68.2 EU/mL) and in UC (median of 57.7 EU/mL) [38]. In adults, a preoperative pANCA titer greater than 100 was shown to be a risk factor for the development of chronic pouchitis after ileal pouch-anal anastomosis [39]. An association between high pANCA titers and both pancolitis and backwash ileitis has also been reported [40].

Beyond phenotypic predictions, pANCA titers have been linked to response to therapy. A retrospective study of 56 UC patients with left-sided disease showed that patients who were pANCA-positive were more likely to have treatment-refractory disease than patients with negative pANCA (90% vs 62%) [41]. In a 2007 study of 100 IBD patients starting infliximab, patients who were pANCA+ and ASCA− had lower early clinical response to infliximab (55% vs 76%). In a pediatric study from 2010, Dubinsky et al. reported that pANCA positivity was independently associated with a primary nonresponse to anti-TNF-α therapy in both CD and UC [42].

Another serological marker specific for UC are antigoblet cell antibodies (GAB) occurring in 15–28% of UC patients. Using appropriate assays, GAB are highly specific (and may be pathognomonic) for UC [5]. Recently PR3-ANCA, which is a marker for granulomatosis with polyangiitis, was found in certain subsets of UC patients and appeared to be associated with liver involvement, PSC, and more extensive disease. However, further studies are needed to confirm these findings [43].

In the multicenter pediatric PROTECT study, anti-CBir1 positivity was found in 19% of patients with UC. Additionally, anti-CBir1 positivity was associated with rectal sparing,

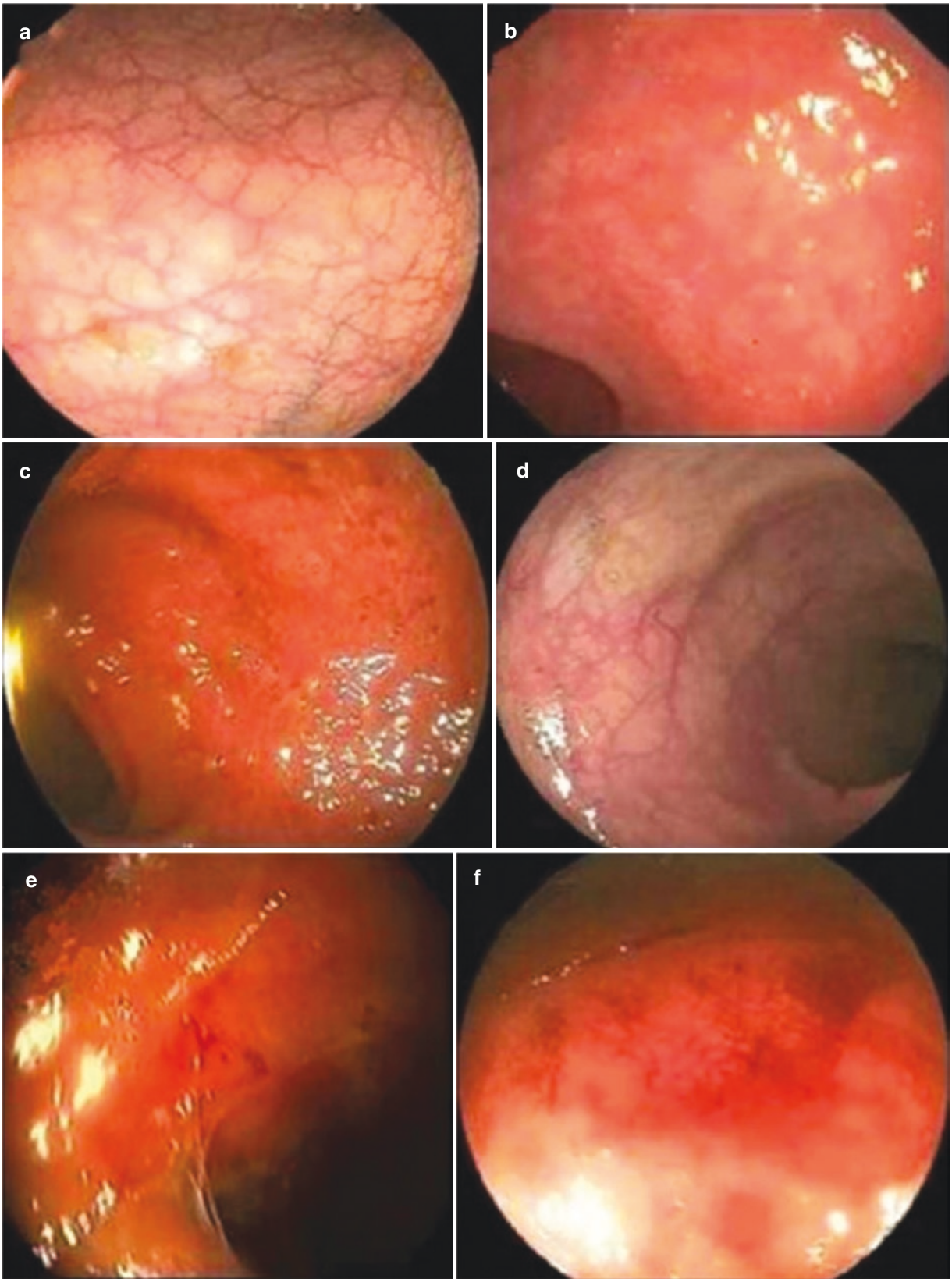

Fig. 30.3 Endoscopic images demonstrating the UCEIS scoring system for: vascular pattern (1–3): (**a**) 1, (**b**) 2, and (**c**) 3; bleeding (1–4): (**d**) 1, (**e**) 2, (**f**) 3, and (**g**) 4; erosions and ulcerations (1–4): (**h**) 1, (**i**) 2, (**j**) 3, and (**k**) 4. (Image courtesy of De Jong et al., "Validation and Investigation of the Operating Characteristics of the Ulcerative Colitis Endoscopic Index of Severity")

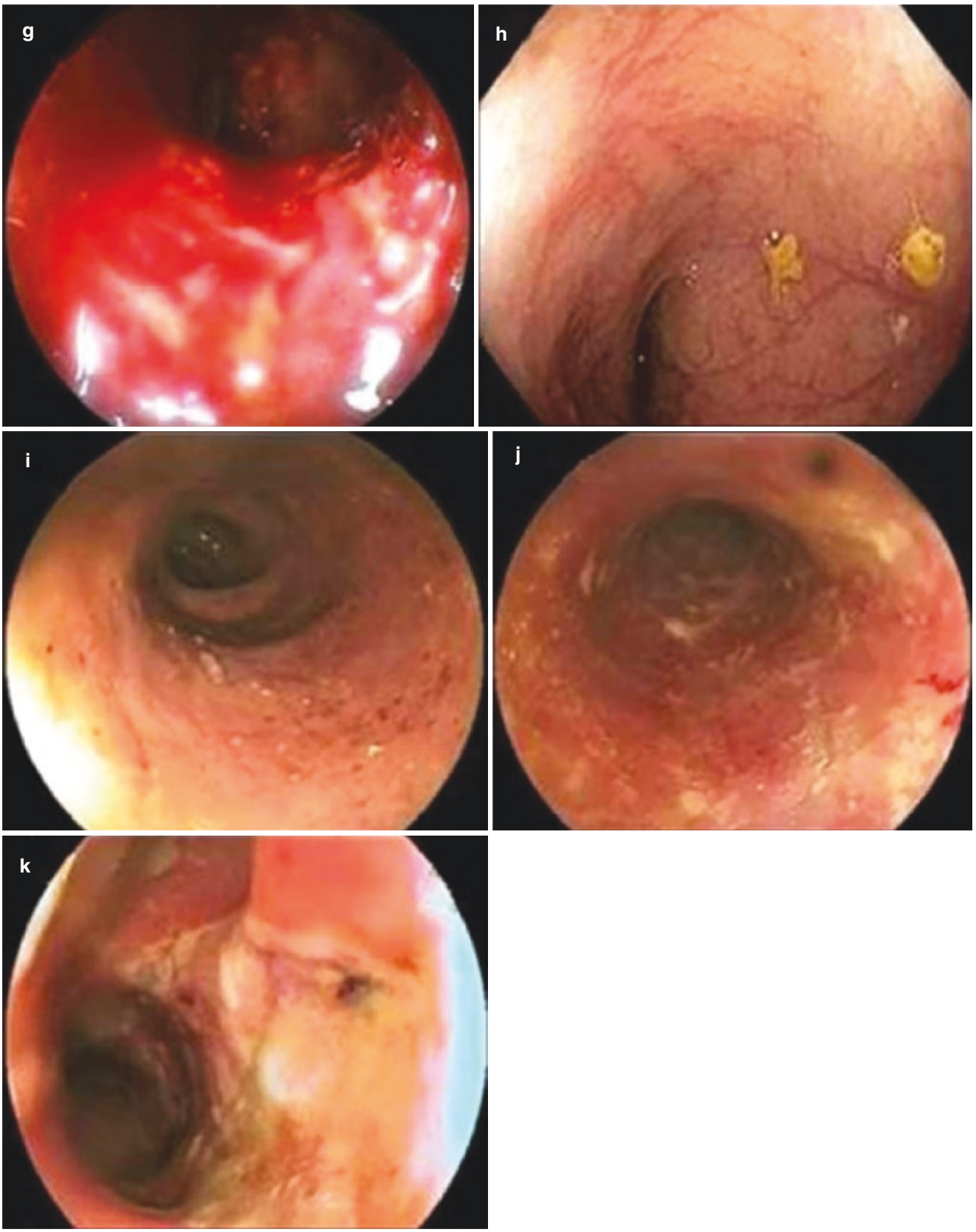

Fig. 30.3 (continued)

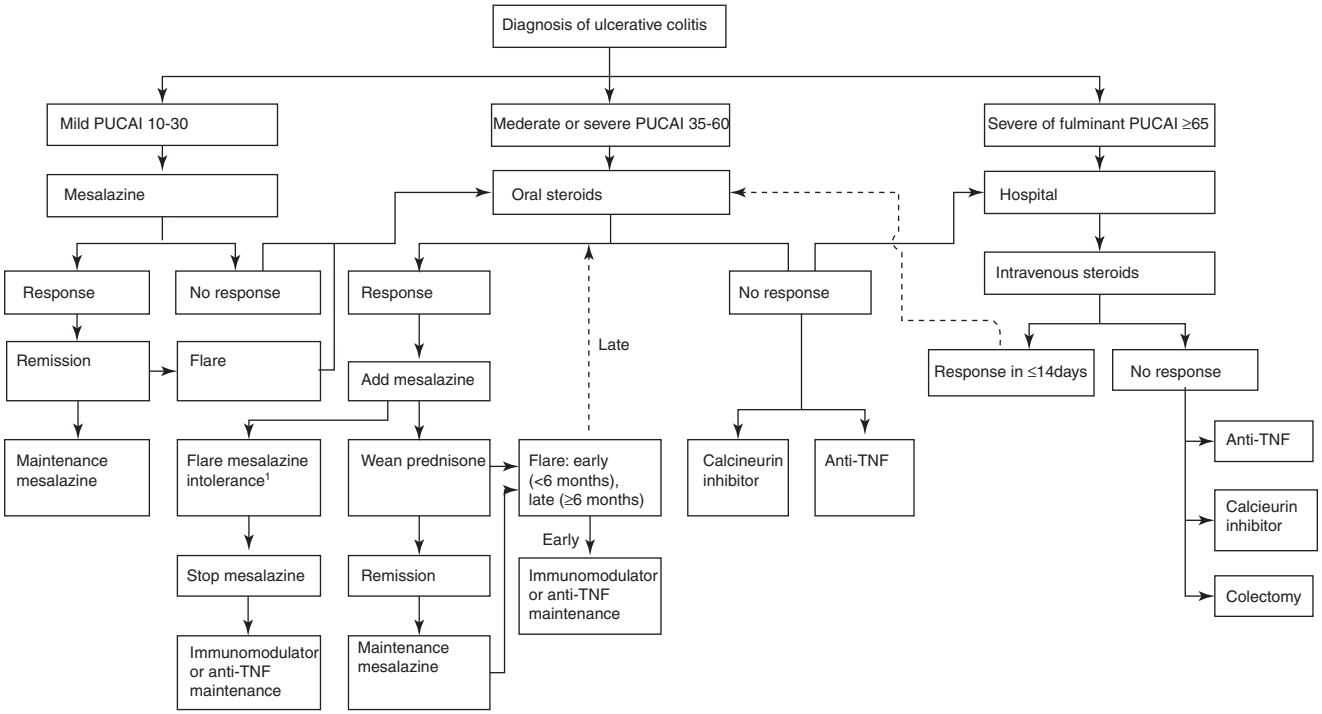

Fig. 30.4 Recommended treatment algorithm for pediatric UC per PROTECT cohort study. (Reprinted with permission from Hyams et al., "Factors associated with early outcomes following standardised therapy in children with ulcerative colitis (PROTECT): a multicentre inception cohort study")

more limited disease extent, less plasma cell infiltrate on rectal biopsy, and lower fecal calprotectin [44].

Activity Indices

There are several UC activity indices for classification and prognosis of UC. For clinical practice, it is sufficient to describe disease activity as mild (up to four bloody stools per day), moderate (four to six bloody stools per day and minimal toxicity), or severe (more than six stools per day and signs of toxicity, such as fever, tachycardia). Fulminant colitis is defined when there are more than 10 bloody stools per day with anemia requiring blood transfusion and colonic dilation on plain abdominal radiographs as a sign of toxic megacolon [5].

A commonly used classification for pediatric UC was developed by the Montreal Working Group. In the "Montreal Classification," disease extent for UC was divided into three categories. The first category (E1) describes patients with proctitis, the second category (E2) describes patients with left-sided disease distal to the splenic flexure, and the last category (E3) describes patients with extensive disease proximal to the splenic flexure. Disease extent was defined using macroscopic appearance rather than histopathologic or radiographic evidence. In 2008, Van Limbergen et al. compared a cohort of children with UC to an adult population with UC, demonstrating that 74.5% of pediatric patients had extensive colitis (E3) based on the Montreal classification, compared to 47% of adult patients [19, 45].

The Montreal classification was further adapted into the "Paris classification for pediatric IBD" in 2011, with greater subdivisions for disease location and age of diagnosis. Using the Paris classification, age groupings are subdivided to distinguish children under age 10 from adolescents between 10 to 17 years of age. The Paris classification includes additional subcategories for disease extent, including E3 (extensive disease distal to the hepatic flexure) and E4 (pancolitis proximal to the hepatic flexure) (See Table 30.1) [46]. E4 pancolitis is the most common phenotype in pediatric UC (57–75%), followed by E2 left-sided UC (10–59%), E1 ulcerative proctitis (5–18%), and E3 extensive disease distal to hepatic flexure (4–13%) [18].

Table 30.1 Differences between Montreal and Paris Classifications for pediatric ulcerative colitis

	Montreal	Paris
Extent	*E1:* Ulcerative proctitis	*E1:* Ulcerative proctitis
	E2: Left-sided UC (distal to splenic flexure)	*E2:* Left-sided UC (distal to splenic flexure)
	E3: Extensive (proximal to splenic flexure)	*E3:* Extensive (hepatic flexure distally)
		E4: Pancolitis (proximal to hepatic flexure)

Table 30.2 Pediatric Ulcerative Colitis Activity Index (PUCAI)

Item	Points
(1) Abdominal pain	
No pain	0
Pain can be ignored	5
Pain cannot be ignored	10
(2) Rectal bleeding	
None	0
Small amount only, in less than 50% of stools	10
Small amount with most stools	20
Large amount (>50% of the stool content)	30
(3) Stool consistency of most stools	
Formed	0
Partially formed	5
Completely unformed	10
(4) Number of stools per 24 hours	
0–2	0
3–5	5
6–8	10
>8	15
(5) Nocturnal stools (any episode causing wakening)	
No	0
Yes	10
(6) Activity level	
No limitation of activity	0
Occasional limitation of activity	5
Severe restricted activity	10
Sum of PULAI (0–85)	

Generally, a PUCAI score < 10 indicates remission, 10–34 mild disease, 35–64 moderate disease and ≥65 severe disease
Reprinted with permission from Siow et al. "*Management of acute severe ulcerative colitis in children*"

The Pediatric Ulcerative Colitis Activity Index (PUCAI) as developed by Turner et al. is a tool that is widely used in children, in part due to its feasibility, validity, and its potential use as a primary outcome measure to reflect disease activity (See Table 30.2). The PUCAI is a noninvasive tool for assessment of UC disease severity consisting of six clinical items: daily abdominal pain, rectal bleeding, stool consistency, number of stools, nocturnal stools, and activity level for a maximum score of 85. Scores under 10 represent remission while a score ≥65 signifies severe disease activity. Cut-off scores for remission, mild, moderate, and severe disease have been shown to have sensitivity and specificity of >90%. PUCAI is furthermore useful for assessing clinical response to treatment as a fall of >20 points signifies improvement due to medical therapy [47].

Cross-Sectional Imaging

Imaging modalities are of limited utility in the setting of acute colitis when a firm diagnosis of UC has been previously established (unless complications such as toxic megacolon are suspected). However, if the diagnosis of CD has yet to be ruled out, imaging of the entire GI tract should be performed to help distinguish between UC and CD [48]. This is also relevant for future surgical planning in children undergoing an elective colectomy to effectively rule out CD [49].

Small bowel follow-through (SBFT) was previously considered the standard of care in evaluation of the small bowel in IBD, but has fallen out of favor because of its limitations, including the length of time required to complete the test and increased radiation exposure. Furthermore, SBFT has been shown to have limited sensitivity for detecting small bowel disease when compared to newer radiographic methods. Enterography, which uses enteral contrast to optimally view the small bowel, is becoming more widely used and has proven to be highly sensitive for identifying inflammation and strictures. It encompasses computed tomography enterography (CTE) and magnetic resonance enterography (MRE). CTE is more commonly used in the adult population; however, given the high dose of radiation associated with this modality, its use has been limited in pediatrics. MRE is therefore replacing other imaging modalities as the preferred method in identifying small bowel disease in children [49].

Infectious Etiologies

Clostridium difficile (*C. difficile*)

Patients with IBD are more susceptible to *C. difficile* infection compared to the general population. Nylund et al. found that children with IBD were 11 times more likely to have a diagnosis of *C. difficile* compared to patients without IBD [50]. *Clostridium difficile* infection has been shown to worsen disease severity, prolong hospital stay, increase the need for parenteral nutrition and blood transfusions, as well as increase colectomy rates and colitis relapses.

Nucleic acid amplification tests (NAATs) such as PCR for *C. difficile* toxin genes have surpassed toxin A and toxin B enzyme immunoassays (EIA) as the preferred method for diagnosing *C. difficile,* having both high sensitivity and specificity [51].

In the setting of acute severe UC (ASUC), treatment with oral vancomycin (40 mg/kg per day orally divided into four doses for 10 days) is preferred due to several adult studies showing greater response rates to vancomycin compared to metronidazole in the setting of ASUC. However, if the patient has a coexisting ileus or toxic megacolon, intravenous metronidazole (30 mg/kg in four divided doses for 10 days) with Vancomycin delivered via enema is the recommended treatment [52].

Cytomegalovirus (CMV) Colitis

Several investigators have demonstrated that CMV infection is detected more often in patients with steroid-refractory UC and is associated with increased rates of colectomy. A CMV infection rate as high as 67% is seen in steroid-refractory UC compared to 33% in patients with steroid-responsive disease [53]. It is unclear whether CMV positivity is a causative factor contributing to disease severity or simply a marker of more severe disease. Serologic testing for CMV is not reliable and the diagnosis is usually made via polymerase chain reaction (PCR) or immunohistochemistry (IHC) of intestinal tissue biopsies. A consensus study recommends that children with steroid-resistant disease undergo flexible sigmoidoscopy and biopsy to exclude CMV infection. The decision to initiate antiviral therapy if CMV is detected should be made in conjunction with infectious disease specialists [52]. Recommended treatment is IV ganciclovir 5 mg/kg twice daily for 14 days, with remission rates as high as 67–100% with treatment [54]. A meta-analysis showed that the risk of colectomy was significantly lower in patients with corticosteroid-refractory UC treated with antivirals as compared to those who did not receive antivirals [55].

In a multicenter retrospective case-controlled study of 56 children with ASUC, CMV-positive patients were found to be more resistant to intravenous corticosteroids compared to CMV negative patients. There was also an increased 12-month risk of colectomy that was statistically significant ($p = 0.045$) in the CMV-positive patients [5, 56].

Complications

Toxic Megacolon

Toxic megacolon (TMC) is characterized by total or segmental nonobstructive colonic dilation with signs and symptoms of systemic toxicity. Although the exact incidence of toxic megacolon in pediatric IBD is not known, previous data reports the incidence around 1–5%. Mortality has been reported as 19–50% in adult patients with TMC; however, data is lacking in children. The most common diagnostic criteria for TMC in adults were introduced by Jalan et al. in 1969, which includes the presence of fever, dehydration, hypotension, an altered level of consciousness, hematologic and biochemical abnormalities, as well as radiographic evidence of a dilated colon [57].

Although radiographic evidence of colonic dilation alone is insufficient to diagnose TMC, one retrospective study noted that a transverse colon diameter of ≥56 mm (>40 mm in children less than 11 years old) was highly suggestive of TMC [58]. Importantly corticosteroids, which are commonly used in the treatment of acute colitis, may mask clinical signs of TMC or intestinal perforation. TMC should be treated quickly with intravenous broad-spectrum antibiotics (i.e., ampicillin, gentamicin, and metronidazole), correction of fluid and electrolyte imbalances, *nil* per os (NPO) status, and avoidance of gut-motility slowing agents (i.e., opioids and antidiarrheal medications). Children with toxic megacolon should be evaluated promptly by surgeons and conservative management should only be considered in stable clinical conditions and in highly specialized centers; urgent colectomy is recommended if there is no clinical improvement within 24 to 72 hours [52, 59].

Acute Severe Colitis

Approximately 25–35% of children with UC will require hospitalization for acute severe ulcerative colitis (ASUC) during a period of 3 years after the initial diagnosis, roughly double the rate seen in adult-onset disease [60]. An acute exacerbation of UC may manifest with clinical relapse accompanied by local or systemic complications such as massive hemorrhage, toxic megacolon, and multiorgan failure; in some cases, this condition is defined as "fulminant colitis."

The best validated and most widely used index for the diagnosis of adult ASUC is the European Crohn's and Colitis Organization (ECCO) adaptation of the 1955 Truelove and Witts' classification, which defines ASUC as an exacerbation of disease with at least six bloody stools daily and one of the following: tachycardia (>90 beats per minute), temperature >37.8 °C, anemia (hemoglobin <10.5 g/dL), or an elevated ESR (>30 mm/h) [61]. However, the use of these criteria has never been validated in children with severe colitis. Instead, PUCAI scoring is more widely used for children, with severe disease defined as a score of at least 65 points. Unlike the Truelove and Witts' classification, which is only useful in diagnosing the acute presentation of disease, the PUCAI can be used to monitor disease severity over time as well as response to treatment [48].

ASUC carries a mortality rate of around 1% primarily from perforation, TMC, and infectious complications. Emergent colectomy is now performed infrequently, with medical therapies being used as first-line therapy in ASUC [48].

With few exceptions, children with ASUC should be admitted to the hospital for immediate evaluation and intensive medical treatment. Intravenous methylprednisolone 1 mg/kg/day (up to 40 mg/day) once daily is recommended as the initial treatment on admission; a higher dose of 1.5 mg/kg/day (up to 60 mg/day) in 1 or 2 divided doses should be reserved in children with more severe presentations or who have failed oral steroids before admission. According to a

recent prospective study of pediatric patients with ASUC, higher doses of IVCS > 1.5 mg/kg/day were not associated with better outcomes [62]. Approximately 65% of patients will respond to IVCS alone.

Intravenous fluid for rehydration and correction of electrolyte imbalances should be provided. Blood transfusions and albumin infusions may also be required. Although traditionally patients were restricted from taking food orally, there is no data to support NPO status in UC. In general, patients should be allowed an oral diet if tolerated, and enteral or parenteral nutrition should be provided if an oral diet is not tolerated or in patients with malnutrition. However, enteral feeding is contraindicated if there is concern for toxic megacolon [59].

Bacterial causes for ASUC should be excluded by stool PCR, including *C. difficile*. In the case of *C. difficile* colitis, oral Vancomycin should be considered as first-line therapy in a patient with ASUC. Given the risk of toxic megacolon, an abdominal X-ray (AXR) should be performed upon admission with a low threshold in patients with abdominal tenderness, abdominal distension, significant pain, and systemic toxicity.

In patients who are not clinically improving after 2–3 days of IVCS therapy, surgical consultation should be pursued for potential colectomy. Additionally, CMV colitis should be excluded in children not responding to 3 days of IVCS with a flexible sigmoidoscopy and mucosal biopsies. Second-line therapy, also known as "rescue therapy" or "salvage therapy," should be initiated on the fifth day of IVCS treatment in children with a PUCAI > 65, as this indicates a nonresponse to steroid therapy. Infliximab is recommended as the second-line medical therapy of choice for anti-TNF-α naïve children failing IVCS, with infliximab continuing as a maintenance treatment after discharge from the hospital. Calcineurin inhibitors such as Cyclosporine and Tacrolimus are alternative second-line medical therapies, but if commenced should be weaned within several months as a "bridge" to thiopurines or another maintenance medication such as vedolizumab.

In general, prompt referral for urgent colectomy is recommended following failure of one second-line medical therapy. Children should not be discharged from the hospital unless the disease is at most mild (PUCAI < 35 points) but preferably closer to remission (PUCAI < 10 points) [59].

In a systematic review, the pooled steroid-refractory rate in ASUC across all pediatric studies was 34%, slightly higher than the pooled 29% rate found in adult studies. Additionally, a child who has ever developed an episode of ASUC is at a higher risk for more refractory disease and future colectomy. The advent of calcineurin inhibitors and infliximab has reduced the short-term colectomy rate from 40–70% to approximately 10–20% in children and the 1-year colectomy rate from ~60% to 18–22%. Among those who fail IVCS treatment, roughly 50–60% of responders to rescue therapy will require colectomy within 1 to 2 years [52, 59].

Antibiotics are not routinely recommended in children with ASUC; however, they may provide clinical efficacy likely through modulation of the microbiome. According to the recent PRASCO randomized controlled trial based in Israel, hospitalized children with ASUC defined by a PUCAI ≥ 65 were randomized to receive antibiotics in addition to IVCS (amoxicillin, vancomycin, metronidazole, and doxycycline/ciprofloxacin) or IVCS alone for 14 days, with the antibiotic arm achieving lower mean PUCAI scores compared to the IVCS only arm. However, long-term outcomes are unknown [63, 64].

Medications

5-Aminosalicylic Acid Agents (5-ASA)

Aminosalicylates or 5-ASAs are the first-line treatment for induction and maintenance of remission, of mild-to-moderate UC. They have a wide range of anti-inflammatory and immunomodulatory properties, including inhibition of 5-lipoxygenase, scavenging of reactive oxygen metabolites, and inhibition of interleukin-1 synthesis. Free 5-ASA is almost completely absorbed from the stomach and proximal small intestine, so sustained-release preparations have been developed to deliver the medication to more distal sites of inflammation.

Sulfasalazine has traditionally been used in UC and is composed of 5-ASA linked to sulfapyridine via a diazo bond that is cleaved by colonic bacteria. Sulfasalazine can be formulated as a liquid and is useful in young children with UC. However, sulfasalazine has several dose-limiting side effects, including headaches, nausea, anorexia, and reversible oligospermia that limit its use. Newer formulations, including mesalamine, olsalazine, and balsalazide, utilize pH- or time-dependent delivery systems to release higher concentrations of 5-ASA at various sites of the small intestine and colon with fewer side effects, thus allowing treatment to be tailored to the location of disease [11, 65].

5-ASA can also be effectively administered in the form of an enema or a suppository for management of distal colonic inflammation [66, 67]. According to a multicenter nonrandomized study of 49 children with ulcerative proctitis treated with a 5-ASA, 500 mg suppository daily for 6 weeks, there were significant reductions in disease activity index scores at weeks 3 and 6 with treatment [68].

The landmark Predicting Response to Standardized Pediatric Colitis Therapy (PROTECT) study was initiated in 2012 to systematically examine the responses of children and adolescents newly diagnosed with mild-moderate UC, who were placed on treatment regimens of mesalamine and

corticosteroids to determine when escalation to additional therapy was required. Interestingly, only 48% of patients treated with mesalamine achieved steroid-free clinical remission at week 12 without the need for treatment escalation with immunomodulators, anti-TNF-α agents, or colectomy [69]. According to the results from the North American pediatric IBD registry, only about 40% (86/213) of children with UC who were treated with 5-ASA within 1 month of diagnosis were in steroid-free clinical remission at one year [70].

Corticosteroids

The use of corticosteroids for the induction of remission in UC was first described in 1955 and since then has been the mainstay for induction of remission in moderate to severe UC. Methylprednisolone or prednisone is used more frequently than hydrocortisone with a suggested dosage of 1–2 mg/kg up to a maximum of 60 mg/day [54]. Response should be seen within 2 weeks at which point the steroids can be tapered. No defined tapering schedule exists, but a common approach is to taper by 5–10 mg per week until reaching 20 mg, then decreasing by 2.5–5 mg per week until completed [11] (Table 30.3).

Children on long-term steroids may have more steroid-related complications than adults (even when adjusted for weight), including delay of linear growth and puberty, osteopenia, acne, glaucoma, and cataracts. Steroid dependency has also been reported to be higher in children than in adults (45% vs 8%, respectively). Strategies to avoid steroid dependency include optimization of 5-ASA, adjuvant therapy with enemas, and escalation to immunosuppressants or biologic agents. Steroid treatment is not advised for maintenance of remission [65].

Oral steroids with minimal systemic activity (due to high first-pass liver metabolism) such as budesonide-multimatrix (MMX) are effective at inducing remission in mild to moderate UC. Given the lower risk for systemic side effects, these drugs may be considered as alternative first-line induction drugs in those failing 5-ASA [11].

Rectal corticosteroids can be tried as a second-line add-on therapy to induce remission in proctitis or left-sided ulcerative colitis. Rectal corticosteroids can be administered as foam formulations that are often better tolerated than enemas by patients with active distal UC [11].

Table 30.3 Medication dosages in pediatric ulcerative colitis

Agent	Dosage .
Corticosteroids	1.0–2.0 mg/kg/day prednisone equivalent
	IV or PO in divided doses (max 40–60 mg)
Sulfasalazine	25–75 mg/kg/day (max 4 g)
Mesalamine	30–60 mg/kg/day (max 4.8 g/day)
Azathioprine	1–2 mg/kg/day
	Adjust dose based on 6-MP metabolite levels
6-Mercaptopurine	1–1.5 mg/kg/day
	Adjust dose based on 6-MP metabolite levels
Cyclosporine	4–8 mg/kg/day IV or PO
	Trough blood levels 200–250 mcg/ml
Tacrolimus	0.15 mg/kg/day PO
	Trough blood levels 10–15 ng/ml
Infliximab	5 mg/kg intravenous infusion at week 0, 2, and 6 for induction dosing, 5 mg/kg every 8 weeks for maintenance dosing
Vedolizumab	300 mg (adult dosing) at week 0, 2, and 6 for induction dosing, 300 mg every 8 weeks for maintenance dosing
Ustekinumab	Induction IV (adult dosing): ≤55 kg–260 mg as single dose, >55 kg to 85 kg–390 mg as single dose
	Maintenance subcutaneous: 90 mg every 8 weeks
Tofacitinib	10 mg twice daily (adult dosing) for induction and 5 mg twice daily for maintenance

Immunosuppressive Therapy

Thiopurines

6-Mercaptopurine (6-MP) and its prodrug azathioprine are purine analogs commonly used in the treatment of steroid-dependent UC or in children with frequent relapses (>2 a year) who have failed aminosalicylates [71]. The therapeutic effect of thiopurines may take up to 10–14 weeks after the start of treatment. The recommended dose is 2.5 mg/kg of azathioprine and 1–1.5 mg/kg of 6-MP in a single daily dose [72]. Previous meta-analyses of adult data concluded that azathioprine is not more effective than placebo for induction of remission in UC but is superior to placebo in preventing relapse [71, 73]. Prospective pediatric studies reported steroid-free remission rates of 49% at 1 year and 72% at 2 years in thiopurine-treated children [74, 75].

Thiopurines are associated with a less favorable safety profile than 5-ASA. TPMT assay (either phenotype or genotype) should be used before starting thiopurines to identify patients who are at risk for dose-dependent myelosuppression, and in whom this drug should either not be used (if homozygous for variant alleles or having very low TPMT activity) or administered at lower doses (if heterozygous for variant alleles or having low TPMT activity). TPMT testing does not, however, replace the need for mandatory monitoring of complete blood counts (CBCs) especially when starting treatment.

Dose-independent adverse reactions include fever, pancreatitis, rashes, arthralgias, nausea, vomiting, and diarrhea, while dose-dependent toxicities included leukopenia (up to 5%), thrombocytopenia, infections, and hepatitis [65]. Hepatosplenic T-cell lymphoma (HSTCL) is a rare but fatal

complication of thiopurine therapy. Of over 40 reported cases of IBD-related HSTCL, the majority of patients received thiopurines, with or without anti-TNF-a, and almost all were males; there are only extremely rare and anecdotal case reports of children with HSTCL who were treated solely with an anti-TNF-α medication [65].

Therapeutic drug monitoring, which involves measuring thiopurine metabolites 6-TGN and 6-MMP, is a way of optimizing drug efficacy and avoiding myelosuppression. Dose adjustments following measurement of metabolites have been shown to increase disease remission rates and prevent relapse [65].

Thiopurines may also be used in "combination therapy" with the biologic infliximab. In a prospective trial of combination therapy with azathioprine and infliximab for UC, combination therapy was found to be more effective than either azathioprine or infliximab therapy alone for induction of steroid-free clinical remission and clinical response. Combination therapy results in lower rates of antibodies to infliximab and results higher infliximab concentrations, which are associated with greater efficacy. However, there was no statistically significant difference in mucosal healing at 16 weeks between combination therapy and infliximab therapy alone [76].

Methotrexate

Methotrexate (MTX) is a potent folic acid antagonist that decreases purine production at the cellular level. At high doses, MTX has antiproliferative and cytotoxic effects by inhibiting the enzyme Dihydrofolate reductase leading to defective DNA synthesis and cell death. At low doses that are commonly used in the treatment for IBD, it functions as an immunomodulator.

The immunomodulatory effect of MTX is poorly understood, but involves increased concentrations of adenosine, inhibition of cellular proliferation and induction of apoptosis, and decreased production of inflammatory mediators such as interleukins and eicosanoids [77].

A previous Cochrane meta-analysis of methotrexate for induction and maintenance therapy in adult UC concluded that there is no evidence supporting its use; however, this conclusion relied on low-quality evidence. In the METEOR double-blind placebo-controlled trial of 111 steroid-dependent adults with UC, although clinical remission was significantly higher in the methotrexate-treated group vs. placebo (42% vs. 24%, respectively), there was no statistically significant difference in steroid-free remission at week 16 between methotrexate and placebo [65, 78], which calls into question its potential efficacy in UC.

Tacrolimus

Tacrolimus, a calcineurin inhibitor, is a macrolide antibiotic isolated from the soil bacterium *Streptomyces tsukubaensis* that blocks IL-2 synthesis and thus inhibits the proliferation of T-cells, clonal expansion, and the production of cytokines involved in the immunological response [79]. It possesses potent immunosuppressive properties and has been used to prevent organ rejection after allogeneic organ transplantation or graft-versus-host disease after hematopoietic stem cell transplantation [80]. Tacrolimus is commonly used in inducing remission in patients with steroid-resistant UC due to its fast onset of action, in order to prevent or delay colectomy. Tacrolimus is also useful as a temporary treatment "bridge" for steroid-dependent patients until a new maintenance therapy takes effect. Long-term use is not recommended due to adverse effects such as nephrotoxicity [79, 81].

Rectal tacrolimus has been reported in children and adults as a successful third-line treatment of ulcerative proctitis. In a recent double-blind placebo-controlled trial, 8/11 adult patients receiving rectal tacrolimus ointment (1.5 mg twice daily) achieved mucosal healing by week 8, compared with 1/10 receiving placebo. Although usually well tolerated, rare toxicity episodes have been reported [65].

Biologics

Infliximab

Since the advent of biologics for the treatment of pediatric UC, there has been a significant reduction in overall 2-year colectomy rates [82]. Tumor necrosis factor alpha (TNF-α) is a potent proinflammatory cytokine found in the serum and inflamed intestinal tissue in IBD and is involved in the pathogenesis of disease. TNF-α is a cofactor in the production of inflammatory cytokines such as IFN-y and IL-2. Infliximab (IFX) is a chimeric monoclonal antibody (75% human, 25% murine) against TNF-α. IFX has been shown to induce clinical, endoscopic, and histologic remission, to reduce hospitalizations and surgery rates, and is a leading therapy for moderate-to-severe pediatric IBD. The efficacy of anti-TNF-α therapies has been widely demonstrated in adult and pediatric patients with UC. The Active Ulcerative Colitis Trial I (ACT I) and ACT II trials clearly showed the efficacy of IFX in achieving clinical remission, clinical response, and mucosal healing. IFX is effective for the induction and maintenance of clinical remission for pediatric UC. The standard regimen for this therapy is 5 mg/kg at weeks 0, 2, and 6, followed by maintenance doses every 8 weeks [83, 84].

Studies in children have shown a pooled long-term success rate of infliximab in UC of 64% and a steroid-free

remission rate of 38% and 21% at 12 and 24 months, respectively, with a likelihood of avoiding colectomy at 2 years of 61% [85].

There has been an increasing interest in more intensified dosing regimens of IFX with greater emphasis on maintaining therapeutic drug levels to improve remission rates. Patients with severe disease often have lower serum albumin and IFX loss in the stool, which are factors known to affect IFX pharmacokinetics [86]. Therapeutic drug monitoring (TDM) is based on measurement of IFX trough serum levels and antibodies to IFX for assistance in clinical decision-making. Reactive TDM has been shown to improve outcomes, with trough levels between 3 and 7 μg/mL being correlated with clinical and endoscopic remission. Conversely, given the chimeric nature of this medication, the development of antibodies to IFX is possible. Positive antibodies to IFX have been associated with immunogenic loss-of-response to therapy, which may require a switch to a different agent or combination therapy with a thiopurine [87].

Adalimumab

Adalimumab (ADA), a fully human monoclonal antibody against TNF-α, is less immunogenic than the chimeric antibody IFX and has been initially proven to be as effective as IFX in CD. The pivotal Ulcerative colitis Long-Term Remission and maintenance with ADA (ULTRA) 1 and 2 clinical trials showed the effectiveness and safety of ADA compared to placebo in inducing and maintaining remission at week 8 and 52 weeks in patients with moderate to severe UC who had failed conventional treatment [88]. The open-label extension study, ULTRA 3, confirmed a favorable long-term safety profile of ADA [89]. ADA is commonly used in clinical practice as an off-label treatment for children with UC. According to a retrospective study by Aloi et al., of 32 children who received ADA after prior IFX treatment (either due to nonresponse or the presence of anti-IFX antibodies), 41% were in steroid-free remission and 28% achieved mucosal healing after 52 weeks [90].

Vedolizumab

Vedolizumab is a humanized α4-β7 integrin antagonist, characterized by a gut-selective mechanism of action and less risk of systemic immunosuppression. By binding to surface-expressed α4-β7 integrin, it inhibits T-cell migration into inflamed intestinal tissue. Its efficacy and safety have been evaluated in numerous studies, mostly in adult patients with moderate to severe UC and CD [91]. In two integrated randomized double-blind placebo-controlled trials of vedolizumab in patients with active UC (GEMINI I), vedolizumab was found to be more effective than placebo for both induction and maintenance of remission. Response rates at week 6 (induction) were 47.1% in the vedolizumab group and 25.5% in the placebo group. At week 52 (maintenance), 41.8% of patients who received vedolizumab every 8 weeks and 44.8% of patients who received vedolizumab every 4 weeks maintained clinical remission, compared to 15.9% in the placebo group [92].

In a phase 3b double-blind randomized trial conducted at 245 centers in 34 countries, vedolizumab was found to be superior to adalimumab in achieving clinical remission and endoscopic improvement, but not in achieving steroid-free remission at week 52 [93]. In a retrospective study of pediatric IBD patients receiving vedolizumab (of which 42% had UC), week 14 remission rates were 76% in patients with UC versus 42% in patients with CD. Additionally, anti-TNF-a naïve patients experienced higher remission rates compared to those with previous anti-TNF-α exposure [94]. The clinical response with vedolizumab is slow compared to anti-TNF-α therapies. While the clinical response compared to placebo may be seen at week 6, peak effect of vedolizumab may not be expected until weeks 10–14 [95], which sometimes necessitates the addition of a treatment "bridge" such as Tacrolimus.

Ustekinumab

Ustekinumab is a monoclonal antibody to the p40 subunit of interleukin-12 (IL-12) and interleukin-23 (IL-23) and has been approved for the treatment of psoriasis, psoriatic arthritis, moderate to severe CD in adults, and more recently in moderate to severe UC in adults. In a phase 3 clinical trial of Ustekinumab in adult patients with moderate-to-severe UC, Ustekinumab was more effective than placebo in achieving induction of clinical remission at 8 weeks and maintenance of remission at 44 weeks. This effect was observed in both biologic-naïve patients and patients who had failed a previous biologic [96]. In a recent single-center retrospective study on the use of Ustekinumab in pediatric IBD in which 8% of the study patients had UC, 90% of patients who were biologic-naïve and 50% of patients who failed a previous biologic achieved steroid-free clinical remission at 52 weeks, indicating the efficacy of this medication in the treatment of IBD [97].

Tofacitinib

Tofacitinib is a newer oral small-molecule Janus Kinase (JAK) inhibitor that is approved in multiple countries for the treatment of moderate to severe biologic-refractory UC. The JAK family comprises four intracellular tyrosine kinases—

JAK1, JAK2, JAK3, and nonreceptor tyrosine-protein kinase 2—that regulate signaling for multiple immune mediators implicated in IBD, including IFN-y and interleukins 2, 4, 6, 7, 9, 12, 15, 21, 23, and 27. Tofacitinib inhibits all JAKs but preferentially inhibits JAK1 and JAK3 [98]. In 2018, tofacitinib was approved for the treatment of adults with moderate to severe UC. It has not yet been approved in pediatric populations [99].

Three phase 3 trials known as OCTAVE investigated the use of tofacitinib in induction and maintenance of remission for UC. In two identical phase 3 trials of induction therapy with tofacitinib in patients with moderate to severe UC (OCTAVE induction 1 and 2), the rates of clinical response, clinical remission, and mucosal healing at 8 weeks were significantly higher in patients who received oral tofacitinib 10 mg twice daily than in patients who received placebo. Tofacitinib was shown to have a rapid onset of action, with significant improvement in partial Mayo score observed as early as 2 weeks into treatment. In a third phase 3 trial (OCTAVE Sustain) which evaluated maintenance therapy with tofacitinib, clinical response, clinical remission, and mucosal healing were maintained at 52 weeks with tofacitinib at a dose of either 5 mg or 10 mg twice daily [98].

Tofacitinib has a similar safety profile compared to biologic agents; however, it is associated with an increased risk of herpes zoster infection. This infection risk is dose-dependent, with 10 mg twice daily being more associated with herpes zoster than 5 mg twice daily [100].

Probiotics and Dietary Therapy

Nutritional therapies for IBD have garnered significant interest due to their limited side effect profile, bowel-sparing nature, and naturalistic approach. An individual's diet is thought to play a key role in IBD development as certain foods have been found to increase proinflammatory cytokines, change intestinal permeability, and affect the composition of the intestinal microbiome. Examples of "pro-inflammatory foods" include animal fats and nondigestible dietary carbohydrates as is typical in "Western diets." Conversely, diets high in fruits, vegetables, and fiber have been shown to be associated with a decreased risk of developing IBD [101]. However, the benefit of nutrition as primary therapy for the treatment of UC has not been proven, and enteral or parenteral nutritional supplementation does not appear to increase remission rates or reduce the need for colectomy in UC [102].

Limited studies have explored the relationship between specialized diets and UC. Gearry et al. retrospectively evaluated low fermentable, oligo-, di-, monosaccharides and polyols (FODMAP) diets in 72 patients with IBD (52 CD and 20 UC) for 3 months. Based on self-report, 70% of patients remained adherent to the diet after 3 months, and symptoms of pain, bloating, and diarrhea improved among those who were adherent to the diet. However, there was no significant reduction in disease activity [103, 104].

One of the more commonly used dietary therapies for IBD is the specific carbohydrate diet (SCD), developed by Dr. Sydney Haas, a pediatrician, in the 1930s to treat patients with celiac disease. The SCD was popularized in the late twentieth century by Elaine Gottschall, after her daughter with UC was successfully treated with SCD by Dr Haas. The SCD diet excludes all grains, sugars (except for honey), processed foods, and dairy, except for specific fermented yogurt and some hard cheeses. It is hypothesized that this diet decreases intestinal inflammation by changing the intestinal microbiome from a proinflammatory state to an anti-inflammatory state [105]. There are currently limited studies formally evaluating SCD and its effectiveness for the treatment of IBD. Patient perception seems to support the use of SCD, with 33% reporting remission 2 months after initiation of SCD and 42% achieving clinical remission at 6 and 12 months. A small retrospective study of pediatric patients demonstrated persistent mucosal disease in patients on a modified SCD (rice, oats, quinoa, and potatoes added to diet), who were otherwise asymptomatic with normal or mildly abnormal labs (including fecal calprotectin). Therefore, despite the positive clinical response seen on a strict SCD, the diet is difficult for many patients to maintain long term, and its efficacy in both serological and histological improvement of disease is unclear [101].

Probiotics have been suggested to be beneficial for induction and maintenance of remission in pediatric UC. Specifically, the probiotic preparation VSL #3 has been shown in a small randomized placebo-controlled trial of 29 patients to be efficacious in active pediatric UC, with 92.8% of patients achieving remission with VSL #3 as well as standard IBD therapy compared to only 36.4% of patients achieving remission with standard IBD therapy alone [106]. In a meta-analysis of 12 randomized controlled trials on UC evaluating the effect of different bacterial strains (VSL #3, Bifidobacteria, and *E. Coli* Nissle) on induction of remission, only VSL #3 showed a significantly increased response rate. However, probiotics had no beneficial effect compared to placebo on maintenance of remission [107].

The ECCO/ESPGHAN consensus guidelines on the management of pediatric UC assert that there is insufficient evidence to recommend routine probiotic use for induction or maintenance of remission. As for pediatric pouchitis, there was a 100% consensus for the usefulness of probiotics in the maintenance of an antibiotic-induced remission in subjects with recurrent or chronic pouchitis [108].

Fecal Microbiota Transplantation

"Dysbiosis," which is characterized by alterations in the composition of the commensal microbiome in a host compared to healthy individuals, is thought to play a major role in the pathogenesis of both UC and CD. There is increasing evidence that the composition of gut microbes in a patient with IBD is different and possibly abnormal and that correction of this abnormality might help control the inflammation seen in patients with IBD. While there have been several controlled studies investigating the efficacy of FMT for adults with UC, relatively few trials have taken place testing FMT in children with UC. These small, uncontrolled studies and case reports have had mixed results [16]. The first published study involved five enemas administered daily to nine UC patients aged 7–21, with 6 of the 9 patients maintaining clinical response at one-month follow-up [109]. In a 2015 case series, a single FMT infusion was administered via nasogastric tube (NGT) to four UC patients, with no clinical response seen [110].

The pediatric literature for FMT remains limited, and conclusions are difficult to draw from such small sample sizes. Yet these studies illustrate several key observations. The failure of FMT delivered via NGT suggests that tailoring modes of FMT delivery to individual patients' disease location and targeting specific "hot spots" may influence patient response rates. Also, microbial material may be degraded from gastric acid exposure during proximal delivery techniques. These studies also demonstrated that UC is best treated by targeting the colon directly with *per rectal* therapy [111]. The translation of this practice to the clinical setting is challenging in most pediatric centers where general anesthesia is required for colonoscopy. This is particularly challenging if multiple FMT administrations are required to maximize efficacy. Lastly, these studies suggest that serial treatment may be required to achieve an appreciable response in IBD patients, in contrast to single or short-course FMT administrations in the treatment of recurrent *C. difficile* infections [16, 111, 112].

Psychosocial Barriers

The chronic nature of UC along with the waxing and waning nature of clinical symptoms can be especially disruptive to children's physical, social, and academic development. Young people with IBD are at an increased risk for behavioral and emotional difficulties compared to healthy children, with depression rates as high as 25% [113, 114]. In pediatric IBD, poorer psychosocial functioning including depression is associated with nonadherence to medical management, risk of relapse, worsened disease activity, and higher healthcare costs. Depressive and anxiety symptoms correlate with disease activity, possibly due in part to the effect of proinflammatory cytokines on the brain, sleep disturbance, and side effects from corticosteroid use [113]. According to a recent study, pediatric patients with UC had significantly more sleep disturbance compared to patients with CD, even without significant differences in nocturnal bowel movements or nocturnal pain [115].

Annual depression screening should be a routine part of IBD care, and clinicians should refer patients for treatment when indicated [113].

Psychotherapy has been shown to be effective in young patients with IBD, including cognitive behavioral therapy (CBT) and supportive nondirective therapy (SNDT). Pharmacotherapy may be helpful as an adjunct to therapy [113, 114].

Nutrition, Growth, and Vitamin D

Malnutrition and growth failure are less common in children with UC compared with patients with CD, but nutritional deficiencies can develop quickly during periods of active disease. In newly diagnosed IBD, short stature has been noted only in CD, and for the most part, children with UC are able to reach their expected adult height. It has been documented that bowel rest with total parenteral nutrition (TPN) or exclusive enteral nutrition (EEN) does not have any therapeutic role in acute UC, although bowel rest can alleviate abdominal pain when severe [34, 116].

Children with IBD are particularly prone to disturbed bone health because of increased circulating inflammatory cytokines, malnutrition, delayed puberty, decreased physical activity, treatment with corticosteroids, and in girls, primary or secondary amenorrhea. Severe osteopenia was present in 3% to 6% of patients with UC compared to 12% to 18% of those with CD. DEXA is the preferred screening tool for bone density measurement in children and adolescents, provided that age- and sex-matched z scores are used. It has been suggested that DEXA be performed in all children newly diagnosed with IBD and repeated in cases of severe disease course, including suboptimal growth velocity, prolonged malnutrition, amenorrhea, delayed puberty, and long or repeated treatments with steroids [34, 117].

Children with IBD are particularly at risk for vitamin D deficiency, and emerging data over the past decade have suggested that vitamin D plays a significant role in both epithelial and immune system dysregulation contributing to IBD pathogenesis. According to the multicenter PROTECT study, vitamin D insufficiency was highly prevalent in children with newly diagnosed UC. Also, free and bioavailable vitamin D, but not total 25(OH) vitamin D, was associated with mean PUCAI scores, indicating that bioavailable vitamin D

may contribute to UC clinical activity. It is widely accepted that vitamin D levels be routinely measured and deficiency treated with vitamin D supplementation, especially in children with decreased BMD. Nutrition support, weight-bearing exercise, and disease control using steroid-sparing strategies are also advocated to improve bone formation [118].

Surgical Therapy

In the majority of cases, medical therapy remains the first-line treatment for UC. However, colectomy may be required for patients with severe or medically refractory disease or in those with colonic dysplasia to prevent the development of colorectal cancer (CRC). Timely surgical intervention in the appropriate setting is imperative to avoid complications of UC. Indications for emergent colectomy in a patient with UC include fulminant colitis or a complication of colitis such as massive hemorrhage, perforation, or toxic megacolon. It is also important to consider colectomy in patients with ASUC who have PUCAI scores > 65 at 11 to 14 days after the start of rescue therapy, as surgery is often unavoidable in these situations. In emergency conditions, the primary surgical strategy is to address the complications of disease by removing the diseased colon and constructing an ileostomy. Elective colectomy should be considered in children with active or steroid-dependent UC despite optimized medical therapy and in those with colonic dysplasia [119].

Except in the setting of emergent colectomy, a complete evaluation should be performed to ensure that there is no evidence of CD prior to colectomy, with small bowel imaging or video capsule endoscopy (VCE), upper endoscopy, and colonoscopy unless there is a contraindication.

Total restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the procedure of choice in patients with adequate anal sphincter function, as it avoids a permanent ileostomy and allows for a preserved body image with a near-normal life for the patient. An IPAA involves a total proctocolectomy with construction of an ileal reservoir anastomosed to the anus. Surgery restores intestinal continuity, preserves sphincter function, and maintains continence. Several types of ileal pouches can be constructed including the J-shaped, S-shaped, W-shaped, and the lateral-lateral pouch. The J-pouch design is the most commonly used for IPAA [120].

The IPAA may be performed in two or three stages, with the use of a one-stage procedure being extremely rare. However, the choice of a two-stage vs. three-stage procedure is more variable [121]. In general, a three-stage procedure (1st stage: subtotal colectomy with ileostomy creation, 2nd stage: proctectomy and IPAA creation, and 3rd stage: stoma closure) [122] is recommended for patients with ASUC, in those treated with high-dose steroids or recent anti-TNF-α therapy, severe malnutrition, or IBD-U. The most common complication of IPAA is pouchitis, which may clinically manifest as diarrhea, tenesmus, and/or constitutional symptoms [121].

Pediatric patients tend to have a higher rate of pouch-related complications than adult patients after IPAA, including pouch stricture and an eventual diagnosis of CD of the pouch. Postoperative pouch-associated hospitalizations and a requirement of postoperative anti-TNF-a biologics were also more frequent among pediatric patients [123]. According to a previous systematic review, there was no association found between number of surgical stages and the following outcomes: pouch failure rates, complication rates, and quality of life [121].

Pouchitis

Nonspecific inflammation of the ileal pouch reservoir, called pouchitis, is the most common complication following IPAA. The symptoms and severity of pouchitis vary from patient to patient, but typically include increased stool frequency and urgency, loose watery stools, abdominal pain, and hematochezia. Duration of pouchitis can be categorized as acute (<4 weeks), chronic (>4 weeks), or recurrent (≥3 episodes of acute pouchitis a year). The reported prevalence of pouchitis in children with UC after IPAA is variable, and data on predictive factors for the development of pouchitis in children are scarce.

A recent multicenter retrospective cohort study from the Pediatric IBD Porto Group of ESPGHAN that included 129 children who underwent IPAA showed that 67% of children developed pouchitis during a median follow-up of 10.5 months from creation of the pouch, including 26% who developed chronic pouchitis. In an older cohort of 399 UC children with a mean age of 18 ± 3 years at colectomy, 36% had at least one episode of acute pouchitis and 9% had pouch failure [124].

Several reports have identified the risk factors of pouchitis as younger age at the time of UC onset, extensive colonic disease or pancolitis, presence of EIMs, preoperative pANCA positivity, and preoperative steroid use. Endoscopic features of pouchitis may include hyperemia, diminished vascular pattern, friability, hemorrhage, and ulcers. Abnormalities may be focal or diffuse and are often more severe in the distal compared to the proximal pouch. Mucosal biopsies typically demonstrate partial to complete villous blunting with crypt hyperplasia and increased mononuclear inflammatory cells and eosinophils in the lamina propria, crypt abscesses, and ulcerations. Mucosal biopsies should be obtained from the pouch and from the afferent ileal loop, but not from the staple line, as erosions and/or ulcers along the staple line do not necessarily indicate pouchitis.

Antibiotic treatment is considered first-line therapy for pouchitis. Only small placebo-controlled trials have been conducted in adult patients to support this practice, and none in children. A 14-day course of ciprofloxacin and/or metronidazole is recommended, with ciprofloxacin possibly being slightly more effective than metronidazole and with fewer adverse events. Chronic and recurrent pouchitis are less responsive to antibiotic therapy. Oral or topical budesonide can be used in refractory cases. Infliximab and adalimumab have also shown efficacy in refractory pouchitis. The probiotic VSL#3 was additionally effective in maintaining remission in adult patients with chronic pouchitis as shown in two double-blind placebo-controlled trials from Italy [124–126].

Cuffitis may cause symptoms similar to those of pouchitis, especially bleeding. The cuff is the remaining rectal mucosa, referred to as a rectal cuff, at the anastomosis between the ileum and anal canal. This area can become inflamed leading to cuffitis, which, in contrast with pouchitis, typically presents with bleeding and can usually be successfully treated with 5-ASA suppositories [11].

Long-Term Prognosis, Colorectal Cancer, and UC

The association between UC and colorectal cancer (CRC) has been a focus of study for many years. IBD-associated CRC (IBD CRC) affects patients at a younger age than sporadic cancer, but the prognosis is similar, with a 5-year survival of approximately 50%.

Chronic inflammation is believed to promote carcinogenesis. The genetic features that lead to sporadic CRC chromosome instability and DNA hypermethylation also occur in colitis-associated CRC. Unlike normal colonic mucosa, cells of the inflamed colonic mucosa have genetic alterations before there is any histological evidence of dysplasia or cancer. Oxidative stress is likely to be involved in carcinogenesis through reactive oxygen and nitrogen species.

In a meta-analysis, UC increased the risk of CRC by 2.4-fold, accounting for an overall occurrence of 1.6% (including sporadic cases) during the first 14 years of follow-up. Male sex, diagnosis at a young age, extensive colitis, presence of PSC, and family history of CRC are all risk factors for the development of CRC [127].

Evidence-based guidelines advise that patients with colitis receive a surveillance colonoscopy 8–10 years after diagnosis, with the interval for further surveillance guided by risk factors. For a subset of patients, annual CRC screening from the onset of disease has been recommended. This includes patients with coexisting PSC and with a first-degree relative diagnosed with CRC before the age of 50. Because CRC lesions in IBD may be harder to detect as they are often flat and multifocal, it has long been advised to take random biopsies every 10 cm throughout the length of the colon, but this approach represents less than 1% of the colonic mucosa and has been shown to miss many dysplasia-associated lesions. Chromoendoscopy is a method in which the colonic mucosa is colored with a dye to enhance mucosal patterns, therefore making it easier to detect dysplastic lesions. Narrow-band imaging, which is now available on most endoscopes, is thought to help visualize dysplastic/neoplastic lesions through enhancing vessels, pit patterns, and soft tissue structures, but it has not been shown to increase identification of dysplastic lesions compared to standard colonoscopy [127, 128].

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Microscopic Colitis

31

Anita Rao and Ranjana Gokhale

Introduction

Microscopic colitis (MC) is an inflammatory condition of the colon marked by chronic or intermittent watery diarrhea with a normal endoscopic appearance but with histological abnormalities [1]. Microscopic colitis is an umbrella term that encompasses two main subtypes: collagenous colitis (CC) and lymphocytic colitis (LC). These subtypes have very similar clinical and epidemiologic features, and the main distinction is made on the basis of histology [2]. Whereas LC is characterized by >20 intraepithelial lymphocytes per 100 colonocytes with mixed inflammation of the lamina propria and normal crypt architecture, CC features a thickened subepithelial collagen band in addition to the inflammatory changes seen in LC. MC affects patients of any age, including children, but most commonly presents in older adults and the elderly with a mean age of 60.7 years [1].

Epidemiology

The reported prevalence of microscopic colitis ranges from 48 to 219 per 100,000 [3], and population-based studies in adults suggest a prevalence of 4–13% in patients investigated for chronic diarrhea [1]. Annual incidence of MC is estimated to be 2.6 to 10.0 per 100,000 person-years, with evidence for an increased incidence over time [4, 5]. The pediatric prevalence of MC remains unknown; however, the youngest reported case is a child 2 years of age [1].

In a pediatric retrospective study by Narla et al, LC was found to be more common than CC, which has also been reported in several adult population-based studies. Also, there was a slight female predominance of MC in this study with a ratio of 3:2, which is lower than what is described for both CC (7:1) and LC (2.4–2.7:1) in most adult reports [1].

Previous epidemiological studies have shown a marked age-dependent rise in the prevalence of MC, especially after the sixth decade of life. Additionally, there is a striking predilection of the disease for female patients [6, 7]. MC typically affects Caucasians, with patients of Indian, East Asian, and Hispanic ethnicity being significantly less affected. Jewish patients tend to be more affected than other ethnic groups [6].

Clinical Findings

MC is characterized by chronic or intermittent watery diarrhea. The severity of diarrhea can range from mild to severe with dehydration and electrolyte abnormalities. Other common symptoms include vomiting, abdominal pain, fecal incontinence, anorexia, abdominal distension, weight loss, and arthralgias [8]. Unlike in adults, MC may manifest in children with atypical symptoms including constipation or alternating constipation and diarrhea [2].

Weight loss is typically mild but can be significant in some cases. MC is not associated with increased mortality, although symptoms can lead to impaired quality of life mostly due to abdominal pain, urgency, and incontinence [3].

Importantly, the symptoms of MC are nonspecific, and many patients with MC actually meet the diagnostic criteria for IBS. Therefore, histologic analysis of colonic biopsies is necessary to distinguish MC from IBS, which is a much more common disorder [9].

Associated Conditions

Although the etiology of MC remains unknown, an autoimmune mechanism is a commonly proposed theory. This rationale is based on the female predominance, frequently

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reported co-occurrence of other autoimmune diseases, possible autoimmune marker positivity, and response to steroid therapy [10, 11].

Concomitant autoimmune conditions, such as Type I diabetes mellitus, thyroid dysfunction, connective tissue disorders, and psoriasis, occur commonly in patients with MC. However, the association between MC and celiac disease is clinically particularly important. In fact, as many as 33% of patients with celiac disease have colonic histologic changes that are consistent with MC. In a large cohort study of patients with celiac disease, 4.3% were diagnosed with MC, 72-fold greater than for patients without celiac disease. MC should therefore be considered in celiac patients who have continued or recurrent diarrhea despite a strict gluten-free diet [9, 10, 12]. Alternatively, celiac disease should be considered in patients with MC who have features of malabsorption, such as significant weight loss, steatorrhea, and unexplained iron deficiency anemia, as well as in those who do not respond to usual therapies. In children, additional associations with MC include collagenous gastritis, immunodeficiency, juvenile scleroderma, eosinophilic gastritis, Crohn's disease, autism, and *Aeromonas hydrophila* infection [10].

There have been reports of patients with MC later developing inflammatory bowel diseases (IBDs) and of patients with IBD developing CC. In a population-based study in Sweden by Khalili et al, there was a significant increase in the risk of incident IBD among adult patients with MC [13]. However, given the small number of cases, these reports could represent random associations of two different diseases [14]. Additionally, many of the histologic features of IBD such as Paneth cell metaplasia and crypt architecture distortion may occur in patients with MC who otherwise have no evidence of IBD [9].

According to two studies of women enrolled in the Nurses' Health Study (NHS) and NHS II, certain lifestyle factors were associated with MC risk. Smoking has been identified as a risk factor for MC, with MC risk increasing with higher pack-years and diminishing following smoking cessation [15]. Surprisingly, unlike many other immune- and metabolic-related disorders, obesity and weight gain since early adulthood were associated with a lower risk of MC based on the results of NHS and NHS II [16].

Pathophysiology

The pathophysiology of MC is not well understood. Several proposed etiologies include autoimmunity/immune dysregulation, reactions to luminal antigens, medications, or infectious insults. It may be that multiple different mechanisms result in similar clinical and histologic features that are labeled as MC [9]. Unfortunately, there is no established animal model available for MC and the current data on pathogenesis are mostly obtained from descriptive studies on patients [17]. Causes and associations of MC in the pediatric population are less clear, in part because of the relative paucity of cases in this age group [1].

Reactions to Luminal Antigens and Dysbiosis

As noted previously, MC is strongly associated with autoimmune disorders such as celiac disease, polyarthritis, and thyroid disorders. In fact, up to 20–60% of patients with LC and 17%–40% of patients with CC have concomitant autoimmune disease [18]. LC-like changes can be induced in patients with celiac disease by a gluten enema [19].

One of the most plausible hypotheses for MC pathogenesis is on the basis of autoimmunity: MC is a chronic inflammatory process against self-antigens unleashed by an initial stimulation (infectious, chemical, or others) in a predisposed individual.

Interestingly, symptoms and histologic changes of MC may resolve with diversion of the fecal stream, which also suggests abnormal immune responses to luminal antigens as well as dysbiosis playing a key role in MC [20]. According to a study by Morgan et al, dysbiosis is the defining feature of the gut microbiome in MC, similar to IBD. However, larger-scale studies are needed to confirm this finding [21].

Genetic Predisposition

In a large genetic study and meta-analysis, ancestral HLA haplotype 8.1 was identified as the major genetic risk factor for CC, and an HLA-DRB1∗04:01 susceptibility allele was identified as being protective. Multiple common susceptibility loci were significantly associated with CC risk, suggesting its polygenic nature [20]. Other studies that have investigated HLA associations have found conflicting results. One study reported an HLA pattern similar to that seen in celiac disease, while another found no HLA association. Abnormal HLA expression on colonocytes also has been described. However, given the conflicting results of these various studies, it is difficult to draw conclusions about the role of HLA haplotypes in MC. Familial cases of MC have been reported, but the infrequency of these observations suggests that genetic predisposition is not a major factor in disease [9].

Infections

Several lines of evidence suggest the possibility of an infectious cause for MC, with bacterial translocation in the gastrointestinal tract being theorized to be a mechanism. Bacterial antigens or toxins are suspected to increase inflam-

matory mediators in the colonic mucosa, leading to increased mucosal permeability, increased cytokines, degradation of the collagen matrix, and dysregulation of intestinal subepithelial myofibroblasts [18].

Also, many patients with MC have acute inflammation on biopsy and/or an acute onset of symptoms similar to gastroenteritis, and patients have been reported to respond to antibiotic therapy. Furthermore, MC has many features in common with "Brainerd diarrhea," a chronic diarrhea thought to be infectious, which is characterized by mucosal lymphocytosis on colonic biopsies [9]. However, no causative organism has yet been identified for MC.

Medication Side Effect

An association between MC and the use of NSAIDs has been reported in some studies but not in others and some patients with MC improve with discontinuation of NSAIDs. Several other drugs also have been implicated as possible causes of MC, including proton pump inhibitors, histamine-2 receptor blockers, selective serotonin reuptake inhibitors, beta-blockers, carbamazepine, and others [18]. One study assessed the strength of evidence that individual medications or classes of medications cause MC and concluded that several drugs had strong evidence. However, there are very few cases of positive drug rechallenge, and the number of cases for any specific drug is small, such that a chance association cannot be excluded. Furthermore, some of the drugs thought to cause MC may simply worsen diarrhea, bringing subclinical cases to diagnosis, but do not actually cause the colitis. Regardless, if a potential case of drug-induced MC is identified, discontinuation of the offending medication may lead to symptom resolution [9].

Malabsorption of Biliary Acids

There is conflicting evidence on the role of biliary acids on the pathogenesis of MC. Colonic infusion of biliary acids in animal models may predispose to colitis, and patients with ileal resection causing malabsorption of biliary acids may have diarrhea. An association between atrophy of ileal villi and MC has also been described. However, small studies conducted with bile acid breath tests have shown little or no evidence of malabsorption of biliary acids in patients with MC. Therefore, the validity of this etiology is still uncertain [9, 22].

Abnormal Collagen Metabolism

Collagen typing studies have identified multiple potential abnormalities in patients with CC. Some studies suggest that the abnormal collagen layer is part of a reparative process in response to chronic inflammation, whereas others suggest a primary abnormality of collagen synthesis. Pericryptal fibroblasts regulate the production and deposition of basement membrane collagen. In CC, they appear to be activated leading to excessive collagen production. However, another study found no evidence for increased collagen synthesis as measured by messenger RNA levels and others have not found elevated levels of fibroblast growth factor.

Transforming growth factor β (TGF-β) may play a role in collagen deposition. This growth factor mediates collagen accumulation and, in one study, patients with CC had increased expression of TGF-β mRNA. Vascular endothelial growth factor (VEGF), another important mediator of fibrosis, appears to be upregulated in patients with CC. Furthermore, treatment with Budesonide reduces VEGF levels, at least in the lamina propria.

It has also been theorized that abnormal collagen deposition may be a secondary process in response to ischemia or another insult. However, this would not account for the inflammatory infiltrate that is seen. Also, the severity of diarrhea is more strongly associated with the degree of inflammation and not with the thickness of the collagen band [9, 22].

Laboratory Findings

Routine laboratory work in patients with MC is typically normal. Some patients with MC have increased erythrocyte sedimentation rate (ESR), low albumin, or positive antinuclear antibodies (ANA) or other markers of autoimmunity, although these markers are neither sensitive nor specific for MC [9]. Small studies have demonstrated that fecal calprotectin was slightly, albeit significantly, higher in those with MC compared to patients without organic causes of diarrhea such as IBS. However, the predictive value was low due to a large overlap of patients with active and quiescent disease as well as normal controls. Fecal calprotectin is not currently recommended for excluding or in monitoring MC [23].

Endoscopic and Histological Findings

In MC, the colonic mucosa generally appears endoscopically normal, although occasionally mild findings such as edema or erythema may be seen. Gross ulcerations suggest an alternate diagnosis, although these can be seen in patients with MC who are taking nonsteroidal anti-inflammatory drugs (NSAIDs).

The classic histologic finding in LC is intraepithelial lymphocytosis (IEL), defined as greater than 20 CD3+ lymphocytes per 100 epithelial cells. The IEL density is usually more prominent in the surface than the crypt epithelium. In addition, biopsies show a mixed infiltrate of acute and

chronic inflammatory cells in the lamina propria (Fig. 31.1). CC has similar inflammatory findings on colonic biopsies, although the IEL infiltrate tends to be less prominent. Therefore, the main distinguishing feature of collagenous colitis is a subepithelial collagen layer thicker than 10 μm, compared with a normal band of approximately 5 μm or less (Fig. 31.2). In addition, biopsies often show surface epithelium damage including detachment of the epithelium in some cases. Biopsies can contain neutrophils, with active cryptitis being reported in a third of patients, although acute inflammation should not dominate the inflammatory infiltrate [9]. Although the histological features of the two subtypes may coexist simultaneously, most pathologists consider the presence of a thickened collagen band to be the major diagnostic determinant and, whenever present, indicate a diagnosis of CC [7]. Morphological abnormalities in MC can be diffuse throughout the colon or can be restricted to one area [4]. There are reports of patients transitioning from one type of MC to the other over time or even having evidence for both types on biopsies from a single colonoscopy. This, in addition to the similar response to treatments, raises the question of whether lymphocytic and collagenous colitis are two separate entities or part of a single disorder. The current approach to these diagnoses is to consider them variants of the same condition [9].

At this time, there is no indication for histological monitoring of disease, as histological assessment of remission and relapse is not standardized and correlation between clinical disease activity and histology is weak. In a study of 283 patients, histological features persisted in postdiagnostic biopsies for up to 1 year in 77% with CC and 64% with LC [23].

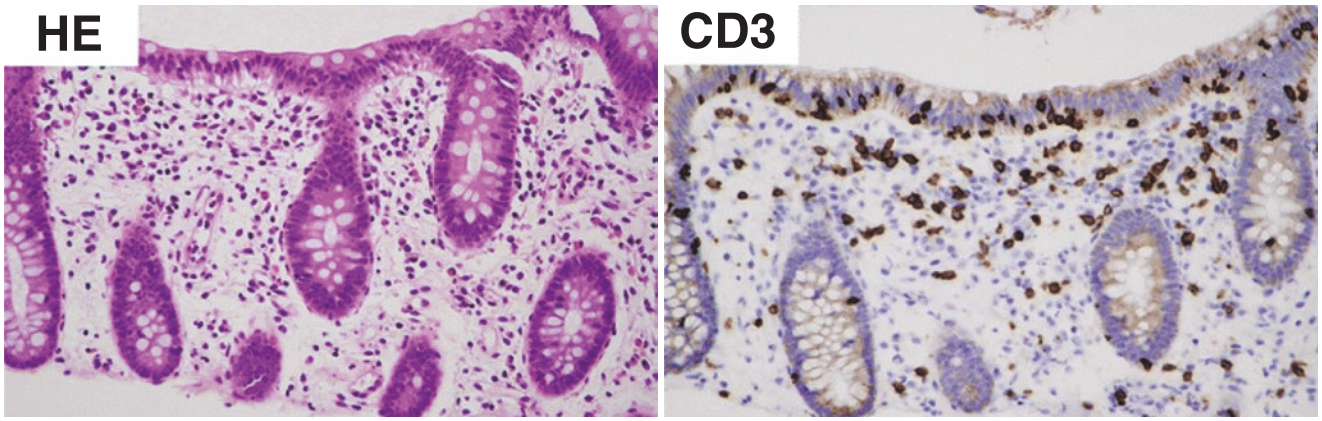

Fig. 31.1 Increase of intraepithelial lymphocytes in lymphocytic colitis. H&E staining (*upper panel*) and CD3 immunostaining (*lower panel*) of the colonic tissue obtained from a lymphocytic colitis patient are shown. Note that immunostaining of CD3 clearly demonstrates the increase of CD3-positive cells in the surface epithelial layer. (Reprinted with permission from Okamoto et al. [48])

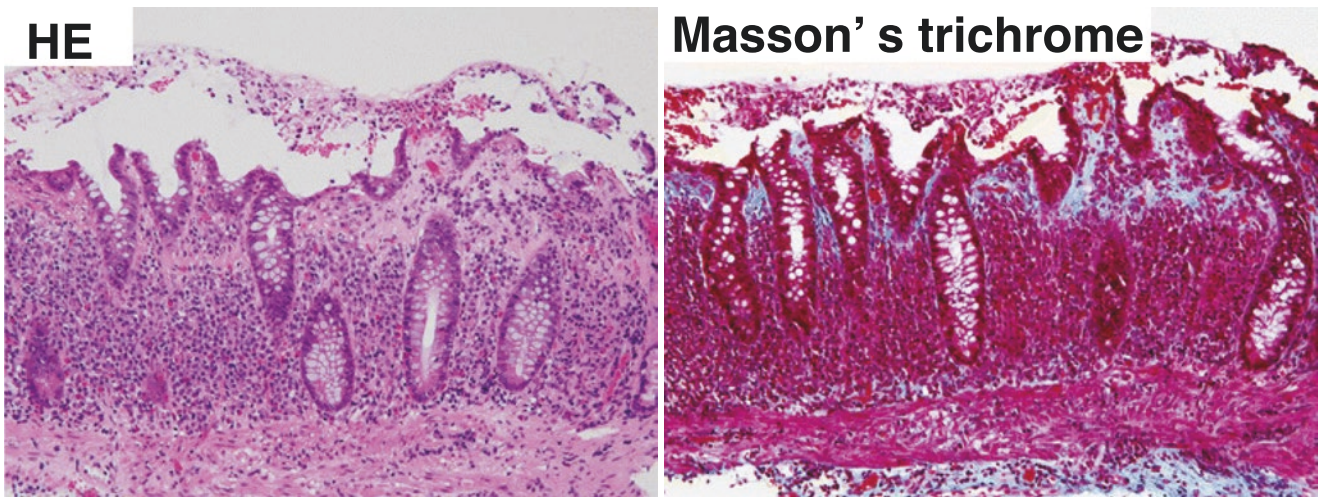

Fig. 31.2 Subepithelial collagen band in collagenous colitis. H&E staining (*upper panel*) and Masson's trichrome staining (*lower panel*) of the colonic tissue obtained from a collagenous colitis patient are shown. Note that thickening of the collagenous layer is clearly observed at the subepithelial area. (Reprinted with permission from Okamoto et al. [48])

Treatment

Unlike other inflammatory colitides, there is no evidence that the persistence of histological inflammation signifies long-term unfavorable outcomes such as colorectal cancer or the need for surgery. The goal of medical therapy is to relieve symptoms and improve quality of life while minimizing drug-related adverse effects [3].

The first step in managing patients with MC is to search for exacerbating factors, including a careful dietary history searching for foods that might contribute to diarrhea, such as dairy in a patient with lactose intolerance or excessive consumption of artificial sweeteners that can lead to diarrhea. It is also important to review the patient's medication list, including over-the-counter products and health food supplements, to search for drugs or other substances that might cause MC or exacerbate diarrhea. In some patients, identification and elimination of these factors may lead to improvement or even resolution of the diarrhea.

Nonspecific antidiarrheal medications such as loperamide or diphenoxylate can be effective in patients with MC and are often used empirically in patients with mild diarrhea. Bismuth subsalicylate may additionally be effective at a dose of 3 tablets (262 mg each) 3 times per day [9]. One open-label study of 13 patients with MC showed clinical remission in 11/13 and histological remission in 9/13 patients with bismuth treatment [24].

For patients with diarrhea who do not respond to antidiarrheals or those with severe symptoms, corticosteroids are typically used. Budesonide, a locally active steroid that undergoes extensive first-pass metabolism in the liver with low systemic exposure, is the best-studied treatment for MC, with four randomized, placebo-controlled induction studies in collagenous colitis [25–28] and three in lymphocytic colitis [4, 29, 30]. In all of these studies, budesonide was superior to placebo for inducing response, with response rates typically in the 80% to 90% range. Budesonide has fewer side effects than prednisone, and unless cost is a significant concern, it is commonly used when corticosteroid therapy is necessary. The efficacy of budesonide is due in part to its potent anti-inflammatory effect in the terminal ileum and proximal colon, [4] with its anti-inflammatory properties also extending to the left colon. Budesonide also improves bile acid malabsorption by upregulating bile acid transporter gene expression in the small intestine [31]. Despite the demonstrated efficacy of budesonide for induction of remission, relapse is common (~70%) when it is discontinued [9].

The American Gastroenterological Association and the European Microscopic Colitis Group both recommend budesonide 9 mg/day for 6–8 weeks as first-line therapy for active MC. In patients who experience clinical relapse after discontinuation of budesonide, low-dose maintenance therapy is recommended, starting at no more than 6 mg/day. Once remission is re-established, the dose is gradually reduced to 3 mg/day, and then to 3 mg every other day. After 6 to 12 months of therapy, another attempt is usually made to discontinue budesonide. If relapse occurs again, budesonide is restarted at the lowest effective dose [4, 9]. Recommendations for long-term therapy are based on studies of budesonide in CC, because no long-term randomized trials of budesonide have been performed in LC [4]. Given the limited systemic availability of budesonide, adverse effects are minimal but may include headaches, nausea, and dizziness. Prolonged steroid use may additionally cause osteoporosis; however, there is little scientific data on this being an issue in patients who are on the medication chronically [32].

Treatment with 5-aminosalicylates such as mesalamine is often considered as a second-line treatment for MC [3]. However, several large uncontrolled studies have reported that only a minority of patients respond to aminosalicylates, and previous studies have shown that Mesalamine 3 g/day administered for 8 weeks was not better than placebo in patients with CC [28] or LC [4].

For patients with steroid-resistant MC, treatment options include bile acid–binding agents or an immunomodulator. Immunomodulators such as azathioprine, 6-mercaptopurine, or methotrexate can be helpful in steroid-dependent or steroid-refractory patients [33–37]. In one single-center retrospective study of patients with severe treatment-resistant MC, azathioprine, and 6-mercaptopurine had a steroid-sparing effect and maintained improvement or remission of diarrhea over a median follow-up of 26 months, with a response rate of 89% [34]. Cholestyramine can be effective, although many patients do not tolerate it because of its texture. Bile-acid binders in tablet form, such as colesevalam or colestipol, might be better tolerated [9]. There have been a few reports of efficacy with the use of antitumor necrosis factor (TNF) therapies such as adalimumab and infliximab in patients with severe steroid-refractory MC [38–40]. Several case series have additionally highlighted the efficacy of Vedolizumab in the treatment of steroid-refractory MC [41–44].

Surgical Treatment Options and Prognosis

Patients rarely require surgery for medically refractory MC. Currently available operations include ileostomy creation with or without a proctocolectomy and ileal pouch anal anastomosis (IPAA), as diversion of the fecal stream normally results in resolution of symptoms [45]. The reported natural history of MC varies considerably. The rate of symptomatic remission ranges from 59% to 93% in patients with LC and 2% to 92% in those with CC. One study reported

spontaneous remission in 15% of patients with CC and treatment-induced remission in another 48% of patients. Only 22% of patients required prolonged therapy. In contrast, clinical trials have reported that only 12% to 40% of patients respond to placebo after 6 to 8 weeks, and an open-label study of steroid therapy reported that 90% of patients required maintenance therapy [46]. In addition, most patients have periods of clinical remission with relapses before lasting clinical remission is achieved [47].

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Vasculitides Including IgA Vasculitis (Henoch–Schönlein Purpura)

32

Karunesh Kumar, Jutta Köglmeier, and Keith J. Lindley

Introduction

The vasculitides are a group of inflammatory disorders of the walls of blood vessels (usually the arteries). They are relatively rare in childhood with the exception of Henoch–Schönlein purpura (HSP) and Kawasaki disease (KD). Vasculitis might be primary or secondary to a number of causes including infections, drugs, hypersensitivity reactions, and connective-tissue disorders. The consequences of arterial inflammation include tissue ischemia and necrosis, giving rise to many of the gastrointestinal (GI) manifestations of vascular inflammation such as pain and bleeding.

Vasculitis is usually classified based on the size of blood vessel(s) involved in the inflammatory process (Table 32.1 and Fig. 32.1). Not all of these are seen in the pediatric age group. The vasculitides associated with GI manifestations in childhood are listed in Table 32.2. These manifestations include abdominal pain (potentially due to bowel ischemia or bowel wall thickening and subacute obstruction), GI blood loss (due to GI ulceration, which can be aphthoid, undermined, or fissure like), diarrhea which is often bloody (due to nonspecific inflammation of the ileum or colon), and an acute abdomen as a consequence of a perforation [1]. Vasculitis subtypes have tendency to involve specific areas of the gastrointestinal tract: Kawasaki Disease (KD) along with Granulomatosis with polyangiitis (GPA) and Eosinophilic granulomatosis with polyangiitis (EGPA) commonly involve the esophagus and oral mucosae; SLE-associated vasculitis, polyarteritis nodosa (PAN), IgA vasculitis (IgAV), EGPA, and GPA may be implicated in

Table 32.1 Classification of the vasculitides

Large-vessel vasculitis (LVV)
Takayasu's arteritis
Giant cell arteritis (GCA)
Medium-vessel vasculitis (MVV)
Polyarteritis nodosa (PAN)
Kawasaki disease (KD)
Small-vessel vasculitis (SVV)
(i) Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)
Eosinophilic granulomatosis with polyangiitis (Churg–Strauss)
Granulomatosis with polyangiitis (Wegener's granulomatosis)
Microscopic polyangiitis
(ii) immune-complex SVV
IgA vasculitis (Henloch–Schönlein; IgAV)
Cryoglobulinemic vasculitis
Hypocomplementemic urticarial vasculitis
Antiglomerular basement membrane disease
Variable-vessel vasculitis (VVV)
Behcet's disease
Cogan's syndrome
Single-organ vasculitis (SOV)
Vasculitis associated with systemic disease
Lupus
Rheumatoid
Sarcoid
Secondary vasculitis
Hepatitis B/hepatitis C
Drugs
Others

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IgA immunoglobulin A

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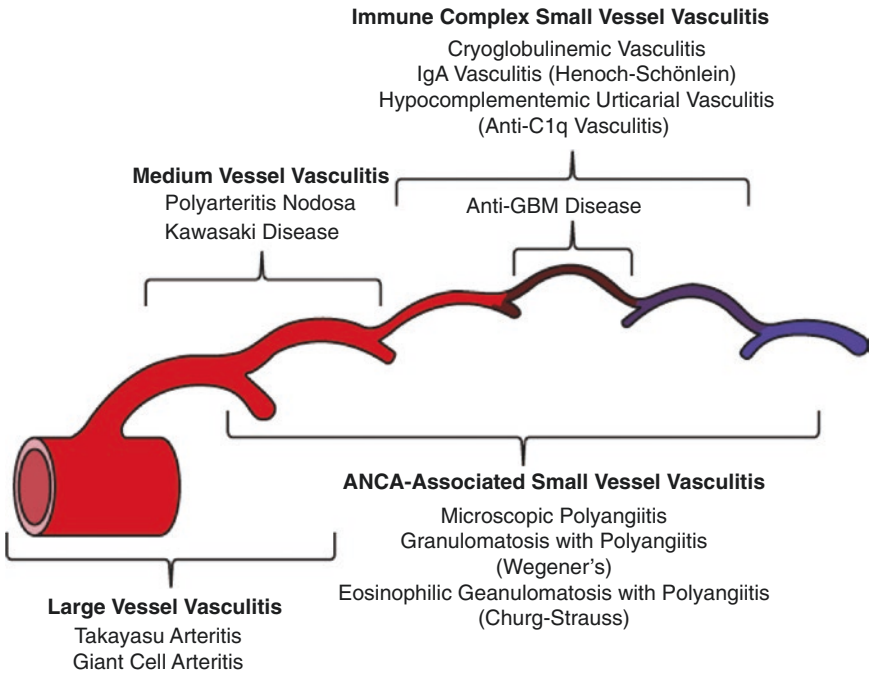

Fig. 32.1 Distribution of vessel involvement by large-vessel vasculitis, medium-vessel vasculitis, and small-vessel vasculitis. Note that there is substantial overlap with respect to arterial involvement, and an important concept is that all three major categories of vasculitis can affect any size artery. Large-vessel vasculitis affects large arteries more often than other vasculitides. Medium-vessel vasculitis predominantly affects medium arteries. Small-vessel vasculitis predominantly affects small vessels, but medium arteries and veins may be affected, although immune-complex small-vessel vasculitis rarely affects arteries. The diagram depicts (from *left* to *right*) aorta, large artery, medium artery, small artery/arteriole, capillary, venule, and vein. Anti-GBM—antiglomerular basement membrane; ANCA—antineutrophil cytoplasmic antibody. *ANCA* Antineutrophil cytoplasmic antibody. (Reprinted with permission John Wiley and Sons/Arthritis & Rheumatism, from Jennette et al. [49])

Table 32.2 Vasculitides associated with gastrointestinal manifestations in childhood

Henoch-Schönlein purpura
Kawasaki disease
Polyarteritis nodosa (PAN)
ANCA-associated vasculitis (AAV)
Behcet's disease
Systemic disease (lupus and rheumatoid)

stomach vasculitis; PAN, Anti-neutrophil cytoplasmic antibody–associated vasculitis (AAV), IgA vasculitis (IgAV), SLE-associated vasculitis, Takayasu's arteritis (TA), and Giant cell arteritis (GCA) have all been associated with intestinal and mesenteric ischemia [2].

IgA Vasculitis (Henoch–Schönlein Purpura)

IgA vasculitis (IgAV), formerly known as HSP, is an immune-complex, nonthrombocytopenic, small-vessel vasculitis, which typically presents acutely [3]. It is the most common vasculitis seen in childhood with an estimated annual incidence of 3–27/100,000 children in the UK [4]. The disease is characterized by a leukocytoclastic vasculitis with deposition of immunoglobulin A1 (IgA1) immune complexes in vascular tissue, principally capillaries and postcapillary venules. The disease is predominantly seen in children aged 3–10 years with the peak incidence aged 4–6 years. The clinical phenotype seems to change with age as older children have more joint symptoms (as in adult-onset IgAV) and younger children more abdominal symptoms. IgAV is more frequent in the autumn/winter months and will commonly follow an infection [5]. Proposed infective triggers include group A beta-hemolytic streptococcus, Parvovirus B19, *Staphylococcus aureus*, and Coxsackie virus to name a few. It has been suggested that the pathogenesis involves the recognition of galactose-deficient IgA1 by antiglycan antibodies and the deposition of these immune complexes in small vessels.

A recent large series suggests that 100% of patients have skin involvement with 60–70% having "palpable purpura" of the lower limbs and buttocks, 66% have arthritis, which is usually symmetrical affecting the knees, ankles, and feet, and 54% have GI involvement usually with lower GI bleeding or abdominal pain but also intussusception, ileal perforation, and pancreatitis [6]. Renal manifestations are seen in 30% and include nephritic or nephrotic syndromes, which can lead to chronic renal failure in a minority of cases. Rash in IgAV is

characteristic: it starts as macules, then these develop into petechiae, raised purpura, or larger ecchymoses in a symmetrical, gravity-dependent manner or on pressure points. The rash may last for up to a week and recur for few months.

Diagnostic criteria include the presence of purpura or petechiae with lower-limb predominance with one of the following: diffuse abdominal pain, acute arthritis or arthralgia, hematuria or proteinuria and any biopsy showing predominant IgA deposition [7].

Described GI complications of HSP include intussusception, bowel ischemia and infarction, intestinal perforation, late stricturing, acute appendicitis, GI hemorrhage (occult and massive), pancreatitis, and gallbladder hydrops. One large series of patients reported abdominal pain in 58% of children and positive stool occult blood (SOB) in 18% [8]. Frank lower-GI bleeding was present in 3%. Plain abdominal radiology frequently showed dilated thickened bowel loops when the SOB was strongly positive, which was also visualized on ultrasound examination. Intussusception, perhaps the most serious GI complication of HSP, was rare (0.5%), although in other series a prevalence of up to 5% is described. The presence of thickened bowel wall on ultrasound might act as a prognostic marker for duration of hospitalization for HSP [9]. Endoscopic findings vary and can include the presence of circumscribed vascular lesions (rather similar to the palpable purpura seen on the skin) and segmental ischemic change (Fig. 32.2) [10].

Recently, it has been proposed that fecal calprotectin might be an early predictor of abdominal type of IgAV as well as a marker of disease severity [11].

It is usual for children to make a complete recovery from HSP with only supportive treatment with the exception of HSP-associated nephritis, which is the cause of end-stage renal failure in up to 2% of children. Recurrence occurs in around 25% of patients, and they may be more common in older children. Most gastrointestinal manifestations are self-limited, but severe abdominal pain, GI hemorrhage, and/or intussusception will require intervention. Corticosteroids as first line have been used: usually oral at 1–2 mg/kg/day for 2 weeks, and then weaned or pulsed IV methylprednisolone 10–30 mg/kg with a maximum of 1 g/day for three consecutive days may be considered [12]. The use of steroids does not seem to protect against the development of nephritis.

Kawasaki Disease

KD is the second commonest childhood vasculitis, which affects about 8/100,000 children younger than 5 years of age annually in the UK with twice as many cases occurring in the United States (25/100,000) and approximately 20 times the incidence in Japan (265/100,000). In common with IgAV, KD is more prevalent in the winter months [13]. The disease affects predominantly medium- and small-sized arteries and is normally self-limiting. However, coronary artery aneurysms are present in 25% of untreated patients and can lead to myocardial infarction or late coronary artery stenosis. In addition to involvement of the coronary arteries, systemic arterial injury can occur.

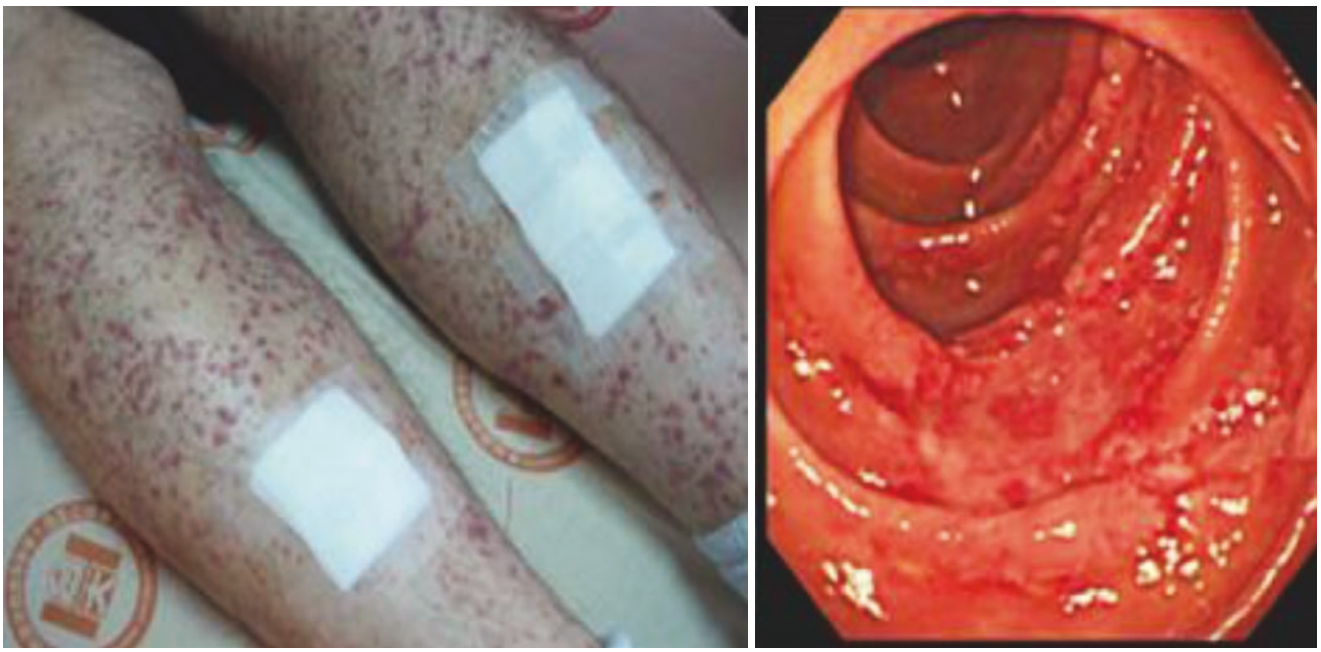

Fig. 32.2 Cutaneous purpura and small bowel hyperemia with ulceration in a young adult with HSP. (Reprinted from Hsu et al. [10], with permission from Elsevier)

The seasonality and clustering of KD support an infectious trigger, although to date no single organism has been identified. The much higher prevalence of disease in Japanese and Korean children supports the notion that genetic predisposition is also an important factor and genome-wide association studies have identified a number of genes associated with disease susceptibility and disease phenotype [13].

Diagnosis of classic KD is clinical, comprising the presence of unremitting fever for 5 days or more plus 4/5 or more of the following features: conjunctivitis; lymphadenopathy; polymorphous rash; changes in lips, tongue or oral mucosa, and involvement of extremities including periungual desquamation (see Table 32.3). A careful history may reveal that ≥1 principal clinical features were present during the illness but resolved by the time of presentation. Patients lacking full clinical features of classic KD may be labeled *incomplete or atypical KD* and if coronary artery abnormalities are detected, the diagnosis of KD is considered confirmed in most cases [13].

Apart from disease defining coronary artery involvement, KD is characterized by inflammation in medium-sized arteries in other organs leading to varied systemic manifestations including hepatitis, pneumonitis, aseptic meningitis, myocarditis, pericarditis, pyuria, pancreatitis, and lymphadenopathy [13].Uncommon features of the disease include gallbladder hydrops, GI ischemia, mononeuritis, nephritis, seizures, and ataxia.

Overall, abdominal symptoms, particularly diarrhea, which can be bloody or nonbloody, are a frequent early feature of KD. Abdominal pain is less frequent [14]. Vasculitic appendicitis, hemorrhagic duodenitis, paralytic ileus, and pseudo-obstruction are also described [15, 16]. Bowel wall edema may be evident as segmental thickening of the bowel wall as may gallbladder hydrops [17].

Inflammatory markers (erythrocyte sedimentation rate, ESR, and C-reactive protein, CRP) are inevitably elevated as is the peripheral blood white blood cell count. Thrombocytosis, which can be very marked, usually occurs in the second week of the disease.

Table 32.3 Diagnostic criteria for Kawasaki disease

Criterion	Description
Fever	Duration of 5 days or more plus 4/5 of the following
1. Conjunctivitis	Bilateral nonpurulent with limbic sparing
2. Lymphadenopathy	Cervical, often >1.5 cm
3. Rash	Polymorphous with no vesicles or crusts
4. Changes in lips/mucus membranes	Red cracked lips, "strawberry" tongue, erythema of oropharynx
5. Changes in extremities	Initially: Erythema and edema of palms and soles
	Later: Periungual desquamation

Early treatment of KD with aspirin and intravenous immunoglobulin (IVIG) reduces the occurrence of coronary artery aneurysms. A single infusion of 2 g/kg IVIG (within 10 days) and an anti-inflammatory dose of aspirin (30–50 mg/kg/day in Europe/Japan and 80–100 mg/kg/day in the United States) will reduce the likelihood of developing coronary artery aneurysms in the majority of patients, although approximately 20% of children are IVIG resistant. The dose of aspirin should be reduced to an antiplatelet dose during the thrombocytosis phase of the illness. Patients who continue to have fevers and an ongoing systemic inflammatory response 48 h post IVIG are likely to be IVIG nonresponders and can be treated with intravenous methyl prednisolone for 5 days followed by 2 weeks of oral prednisolone or perhaps anti-tumor necrosis factor (TNF) antibodies [13].

Systemic Polyarteritis Nodosa

Polyarteritis nodosa (PAN) is a necrotizing arteritis of medium and/or small arteries, which has been subclassified into systemic PAN and cutaneous polyarteritis. While systemic PAN is generally severe and cutaneous polyarteritis relatively benign, the cutaneous form can go on to develop features of multiorgan involvement. The presenting features of PAN can be very nonspecific and are known to affect a number of systems notably the skin, musculoskeletal system, kidneys, and GI tract. A recent pediatric series documents the most common presenting features of PAN as fever (87%), myalgia (83%), arthralgia/arthritis (75%), weight loss of >5% of body weight (64%), fatigue (62%), livedo reticularis (49%), and abdominal pain (41%). In this series, 59% had GI symptoms comprising abdominal pain (49%), blood in the stools (10%), and bowel ischemia/perforation (8%) [18]. The diagnosis may be delayed as the symptoms are so nonspecific. GI bleeding can be massive, especially when it arises from a Dieulafoy lesion, a submucosal vascular abnormality with a prominent tortuous artery with/without aneurysm formation [19, 20]. Ulcers, which are circumscribed and well demarcated, may also be evident (Fig. 32.3a, b). Following remission induction in PAN, the onset of GI symptoms is a major clinical predictor of clinical relapse. Other symptoms seen in PAN less frequently include cardiac, respiratory, and neurological manifestations.

GI symptoms are generally attributable to ischemia, which can lead to infarction, perforation, or stricture [21] [22]. The systemic vasculitides can also be associated with an ischemic colitis or a nonspecific colitis that can mimic inflammatory bowel disease [23].

The diagnosis of PAN is usually made through a combination of clinical, histopathological, and arteriographic features and relies on one mandatory criterion plus one of five secondary criteria. Mandatory criteria comprise fibrinoid

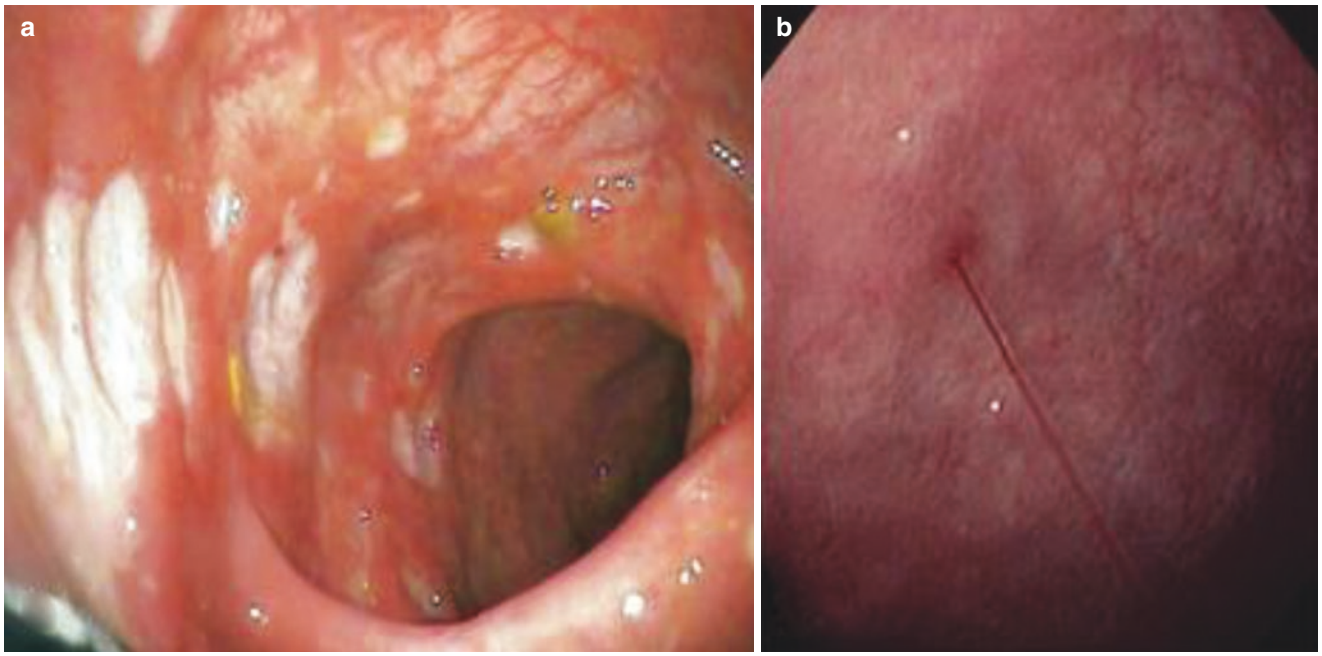

Fig. 32.3 (**a**) Punched-out ulcers with well-demarcated edges in the colon in PAN. Ulcers are typically shallow and irregular often with surrounding erythema. (Reprinted from Vavricka SR et al. Clin Gastroenterol Hepatol 2007; 5(11): A22, with permission from Elsevier). (**b**) Bleeding Dieulafoy lesion in the stomach in a case of PAN. (Reprinted from Maeda et al. [20], with permission from Elsevier)

necrosis of the walls of medium-sized arteries from an affected organ together with an inflammatory response in the adjacent vessel wall, which is characteristic of PAN or angiographic abnormalities (aneurysm, stenosis/segmental narrowing, or occlusion/pruning) of medium or small arteries. Secondary criteria include (i) characteristic skin involvement, (ii) myalgia or muscle tenderness, (iii) hypertension, (iv) peripheral neuropathy/mononeuritis, and (v) renal involvement. Renal, hepatic, and mesenteric arteriography is commonly used as a diagnostic tool in children which overall has a sensitivity of 94% for diagnosing PAN (Fig. 32.4). The microaneurysms that are the hallmark lesions of PAN can be frequently detected in hepatic, splenic, and mesenteric arteries as well as the renal arteries.

There are many differential diagnoses of PAN in children; it is particularly important to exclude monogenic vasculitides such as deficiency of adenosine deaminase type 2 and other autoinflammatory syndromes [24]. ANCA is typically negative in PAN.

A detailed discussion of treatment is beyond the scope of this chapter. Historically, this has involved remission induction with pulsed intravenous methyl prednisolone often with cyclophosphamide and antiplatelet agents followed by maintenance therapy with low-dose steroids and a steroid-sparing agent (usually azathioprine). Biologics may be considered in patients who fail to respond to standard therapy or where concern exists regarding cumulative cyclophosphamide dose; either TNF-alpha or IL-6 blockade or B cell-depleting agents may be considered [24].

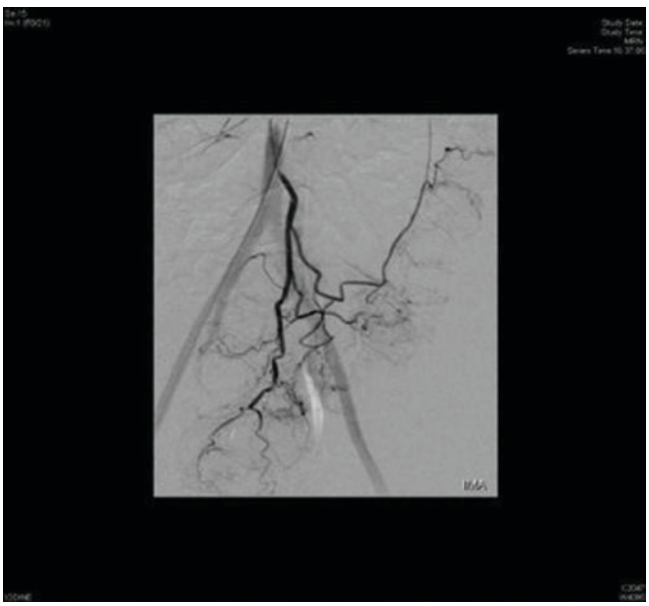

Fig. 32.4 Inferior mesenteric artery angiogram showing vessel caliber changes and aneurysms characteristic of polyarteritis nodosa

Behçet Disease

Behçet disease (BD) is rare in childhood (2/100,000 in Europe). Behçet described a triad of aphthous stomatitis, genital ulceration, and uveitis. It is a variable-vessel vasculitis, which can affect veins and arteries of any size. Three of six items are required to classify a patient as having pediatric

BD: recurrent oral aphthosis (three attacks/year), genital ulceration or aphthosis, skin involvement (necrotic folliculitis, acneiform lesions, erythema nodosum), ocular involvement (anterior uveitis, posterior uveitis, retinal vasculitis), neurological signs (with the exception of isolated headaches), vascular signs (venous thrombosis, arterial thrombosis, arterial aneurysm) [25].

BD is extremely heterogeneous, affecting multiple organ systems with distinct geographical variations in symptoms. For example, GI manifestations are more prevalent in the Far East, while vascular manifestations are seen in the eastern Mediterranean. GI symptoms may be seen in up to 40%, and they are usually isolated abdominal pain or discomfort. Rare features are digestive aphthae, bleeding, and perforation [25]. Vascular manifestations include venous thromboembolism, arterial stenosis, aneurysms, and occlusions. Neuro-Behçets can present with headache, papilledema, central venous sinus thrombosis, and/or brain parenchymal disease causing seizures and focal neurological abnormalities [26].

ANCA-Associated Vasculitis (AAVs)

ANCA-associated vasculitides (AAVs) are multisystemic diseases and include granulomatosis with polyangiitis (GPA, earlier Wegener's granulomatosis), eosinophilic granulomatosis with polyangiitis (EGPA, earlier Churg-Strauss syndrome), and microscopic polyangitiis (MPA). Histopathologically, AAV is characterized by necrotizing changes (granulomatous lesions in GPA and eosinophil-rich granulomatous inflammation in EGPA) in small blood vessels (i.e., capillaries, venules, and arterioles), with few or no immune deposits, and associated with the presence of circulating ANCA autoantibodies that are usually directed against myeloperoxidase (MPO) or proteinase 3 (PR3) [27]. Tissue biopsies are crucial for diagnosis though may not be always necessary. Renal biopsy in case of renal involvement remains the gold standard for diagnosis. Treatment remains based on adult approach comprising remission-induction and remission-maintenance phases. Combination of glucocorticoids and either cyclophosphamide (CYC) or rituximab (RTX) is recommended in induction. Maintenance is recommended with Azathioprine (AZA) or MTX in combination with glucocorticoid. Leflunomide (LEF) or MMF may also be considered as an alternative agent.

Granulomatosis with Polyangiitis (GPA)—Formerly Wegener's Granulomatosis

GPA is one of the antineutrophil cytoplasmic antibody (ANCA)–associated vasculitides (AAV) and is a necrotizing granulomatous vasculitis of small blood vessels [27–29]. The main features of the disease are due to a widespread small-vessel vasculitis with clinical manifestations of a necrotizing glomerulonephritis and respiratory tract granulomata dominating the clinical picture. GI involvement has been described in 6–36% of pediatric patients and may present with abdominal pain; mucosal ulceration of the esophagus, small or large bowel; GI perforation and rarely colitis and catastrophic GI hemorrhage or infarction of the gall bladder, intestine and colon. cANCA positivity is seen in up to 70% of these children.

Eosinophilic Granulomatosis with Polyangiitis (EGPA)

This is another of the AAV, which is extremely rare. ANCA (usually pANCA) is positive in about 30% of cases. Histopathological features are those of a small-vessel (arteries and veins) granulomatous vasculitis with an eosinophil-rich inflammatory infiltrate. In addition, there is an eosinophil-rich granulomatous infiltrate of the respiratory tract and usually a history of asthma, allergic rhinitis, and peripheral blood eosinophilia.

GI-tract involvement is seen in 40–60% of patients causing symptoms of pain (ischemic), ulceration, perforation, and bleeding [27–29]. Independently of vascular involvement, eosinophil-rich inflammation of the GI mucosa can result in symptoms such as diarrhea and perhaps pain. Forty percent of children have gastroenterological symptoms at presentation [30]. The pediatric disease is usually steroid responsive but subject to relapse.

Microscopic Polyangiitis (MPA)

Microscopic polyangiitis is a necrotizing vasculitis of small vessels in which up to 65% of patients are perinuclear ANCA (pANCA) positive. It is uncommon in childhood [31]. The classical presentation is with rapidly progressive glomerulonephritis and alveolar hemorrhage [32]. In common with the other AAV, pain is a dominant GI symptom, although GI blood loss, cholecystitis, ischemic colitis, and bowel perforation are described. GI manifestations can be seen in 11–58% of pediatric patients [27–29].

Single-Organ Vasculitis (SOV)

Systemic vasculitis occurs when vascular inflammation involves multiple territories or organs. In single-organ vasculitis (SOV), the inflammation is restricted to a single organ or part of that organ. By definition, there has to be a

lack of spread outside the single organ for at least 6 months. SOV is known to affect the GI tract and may affect small-, medium-, or large-sized arteries. The condition is not well described in children and is more commonly part of PAN (e.g., appendiceal vasculitis). Patients with gastrointestinal SOV may present with an acute abdomen, and diagnosis may be made based on histological findings after the surgery.

Takayasu Arteritis (TA)

Takayasu arteritis (TA) is a vasculitis predominantly affecting large vessels (mainly the aorta and its main branches). It is most commonly seen in Asia and rarely encountered in the pediatric age group. Diagnosis is based on angiographic abnormalities showing aneurysm/dilatation (mandatory criterion) plus one of the five following criteria: pulse deficit or claudication, four limbs BP discrepancy, bruits, hypertension, and acute phase reactant [7]. Girls are more often affected than boys. Patients frequently complain about headache, abdominal pain, limb claudication, myalgia, arthralgia, and fever. Weight loss is said to occur in about 10% of affected individuals. GI manifestations are rare and occur mainly as ischemic changes due to large involvement in small or large intestine, spleen, or, more rarely, liver. Cases of TA coexisting with IBD have been reported in adult series.

Systemic Lupus Erythematosus–Associated Vasculitis

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease. The presence of anti–double-stranded DNA (anti-dsDNA) antibodies in the blood is a highly specific test for SLE being present in 70–80% with titers of antibody tending to mirror disease activity. Other pathogenic autoantibodies in SLE include antibodies to nucleosomes, Ro (a ribonucleoprotein component), La (an RNA-binding protein), C1q (complement), phospholipids, and the *N*-methyl-D-aspartic acid (NMDA) receptor. Many of these antigens are expressed on the cell surface during the process of apoptosis, and it has been hypothesized that abnormalities of the apoptotic pathway, which are ubiquitous in SLE, are important in the genesis of pathological autoantibodies [33].

Systemic lupus can affect the GI tract causing chronic, nonspecific mucosal inflammation, mucosal ulceration, or vasculitis resulting in mesenteric/GI ischemia (Fig. 32.5a, b). The three possible manifestations of lupus enteritis include lupus mesenteric vasculitis, intestinal pseudo-obstruction, and/or protein losing enteropathy [34]. Gastrointestinal manifestations directly attributable to SLE affect one-fourth of adult patients and vasculitis (usually cutaneous) can be seen in similar number of patients. Commonly, GI symptoms may be because of infection or drug side effects. GIS manifestations has been observed in up to 27.5% children in some series.

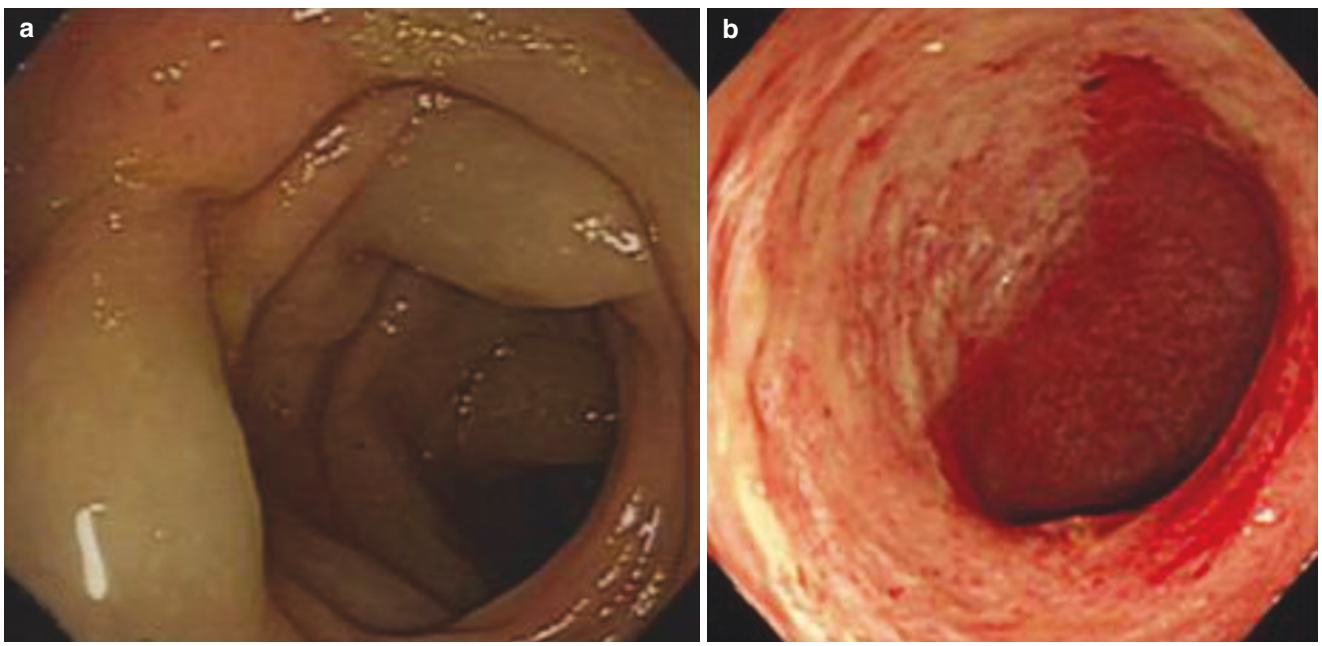

Fig. 32.5 (**a**) Mucosal edema in association with nonspecific inflammation in SLE. (**b**) Colonic mucosal ulceration in SLE. (Reprinted from Lee CK et al. Gastrointest Endosc 2010;72(3): 618–9, with permission from Elsevier)

Abdominal pain is present in 8–40% of published series of individuals with SLE; in children, it is most commonly (32%) due to lupus-associated mesenteric vasculitis [35]. Other causes of pain include pancreatitis (10%), appendicitis (7.5%), and cholecystitis (6%). Patients with vasculitis usually have high SLA disease activity scores (SLEDAI) but do not commonly have substantially elevated inflammatory markers.

The clinical presentation of lupus-associated mesenteric vasculitis commonly mirrors an acute surgical abdomen. It is suggested that individuals with SLE, high SLEDAI, and an "acute abdomen" undergo imaging to look for evidence of vascular compromise before any surgical intervention (Fig. 32.6).

Juvenile Dermatomyositis (JDM)

In juvenile dermatomyositis (JDM), vasculitis affects striated muscle, the skin, subcutaneous tissues, and the GI tract. Children develop weakness of the muscles of the neck, shoulders, and hips, leading to difficulties with swallowing, getting up from sitting, or climbing stairs. Gastrointestinal involvement occurs in 22% to 37% of JDM patients. GI manifestations include dysmotility (dysphagia), vasculitis with associated malabsorption, and other more severe features of GI vasculopathy. Vasculopathy may present with abdominal pain, rectal bleeding, intestinal ischemia, pneumatosis, or life-threatening perforation [36]. GI bleeding and perforation can be catastrophic and are a major cause of death [37].

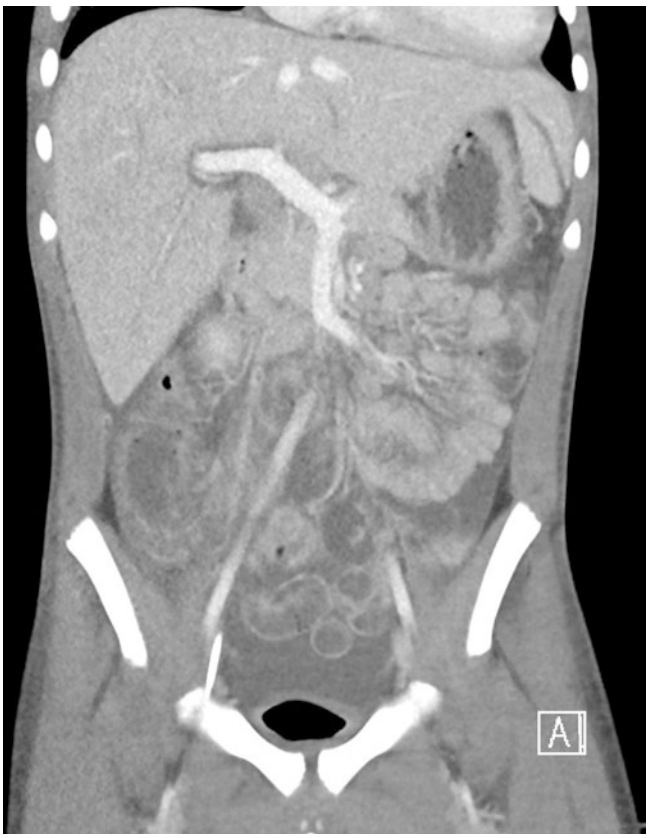

Fig. 32.6 CT scan of patient with PIMS-TS demonstrating mural thickening of the right colon and ascites

Rheumatoid-Associated Vasculitis

Rheumatoid arthritis (RA) is another systemic inflammatory disorder with articular and extra-articular manifestations. Rheumatoid vasculitis (RV) is a systemic necrotizing vasculitis affecting small- and medium-sized arteries, which is clinically extremely heterogenous and can affect skin, nerves, and abdominal viscera. RV is usually seen in long-standing disease and when there are other extra-articular manifestations, and these patients are always rheumatoid factor positive. It is usual to find raised inflammatory markers (ESR and CRP), polyclonal hypergammaglobulinemia, and often hypocomplementemia. GI involvement is rare in children, but, in common with other vasculitides, can result in ischemia, perforation, and hemorrhage [38].

Pediatric Inflammatory Multisystem Syndrome: Temporally Associated with SARS-CoV 2 (PIMS-TS)

In the spring of 2020, the first wave of the COVID 19 pandemic, caused by the SARS-CoV 2 coronavirus, caused over a million deaths worldwide within 9 months. Children, in general, are not severely affected by SARS-CoV 2 infection but rarely children go on to develop a multisystem inflammatory response developing a syndrome, which shares features with Kawasaki disease and toxic shock syndrome. While not usually the presenting complaint abdominal symptoms / signs are prevalent within this group of children and can be a source of diagnostic confusion.

The RCPCH (UK) has adopted the following definition in its guidance for health professionals [39]:

1. A child presenting with persistent fever, inflammation (neutrophilia, elevated CRP and lymphopenia), and evidence of single or multiorgan dysfunction (shock, cardiac, respiratory, renal, gastrointestinal, or neurological disorder) with additional features (Table 32.4). This may include children fulfilling full or partial criteria for Kawasaki disease.
2. Exclusion of any other microbial cause, including bacterial sepsis, staphylococcal or streptococcal shock syndromes, infections associated with myocarditis such as enterovirus.
3. SARS-CoV-2 PCR testing may be positive or negative.

Table 32.4 Clinical and laboratory features of PIMS-TS

Clinical:
All:
Persistent fever >38.5 °C
Most:
Oxygen requirement
Hypotension
Some:
Abdominal pain
Confusion
Conjunctivitis
Cough
Diarrhea
Headache
Lymphadenopathy
Mucous membrane changes
Neck swelling
Rash
Respiratory symptoms
Sore throat
Swollen hands and feet
Syncope
Vomiting
Laboratory findings:
All:
Abnormal fibrinogen
Absence of potential causative organisms (other than SARS-CoV-2)
High CRP
High D-dimers
High ferritin
Hypoalbuminemia
Lymphopenia
Neutrophilia in most (normal in some)
Some:
Acute kidney injury
Anemia
Coagulopathy
High IL-10
High IL-6
Proteinuria
Raised CK
Raised LDH
Raised triglycerides
Raised troponin
Thrombocytopenia
Transaminitis
Imaging:
Echo and ECG—Myocarditis, valvulitis, pericardial effusion, coronary artery dilatation
Abdominal US—Colitis, ileitis, lymphadenopathy, ascites, hepatosplenomegaly

Adapted from RCPCH Guidance (Ref. [39])

Other definitions have been adopted in other countries / continents although all are broadly similar. For example, the Centers for Disease Control and Prevention (CDC) in the United States has termed the condition as multisystem inflammatory syndrome in children (MIS-C) [40]. The reader is referred elsewhere for further information regarding this rapidly evolving field [41].

Notably KD and PIMS-TS have quite a different epidemiology. For example, KD is seen most commonly in children aged <5 years, whereas PIMS-TS is seen predominantly in older children and adolescents. PIMS-TS is seen most frequently in children with a Black African heritage, whereas KD is most common in Asians.

Common clinical features include fever, gastrointestinal symptoms, rash (variable distribution including limbs, face, generalized), and less commonly conjunctivitis [42]. A high proportion present in shock requiring early transfer to an intensive care setting.

Among the first case series to be reported in the UK is a cohort of 15 patients presenting to a tertiary Hospital Cardiology service [43]. The patients were predominantly male and non-Caucasian with a median age of 8.8 years. Many had either a history of positive SARS-Cov 2 PCR or positive combined IgG, IgA, IgM serology. All had pyrexia for a median of 5 days at presentation and 87% had gastrointestinal symptoms. Another series of 35 children [44] found 87% to have gastrointestinal symptoms including abdominal pain, vomiting, and diarrhea. In this series, approximately 50% had abnormal abdominal ultrasonography with findings including RIF inflammatory changes, mesenteric lymphadenopathy, and bowel wall thickening of the ileum and / or right colon. A further series of children with prominent gastrointestinal symptoms had similar findings on imaging [45]. It is the author's experience that with treatment of the PIMS-TS/MIS-C, these bowel/intra-abdominal findings resolve completely without the need for invasive investigation.

Investigation of Children with Suspected GI Vasculitic Disorders

The diagnosis of vasculitis can be challenging because of the heterogeneity of possible presentations and the diagnosis is often delayed. A high index of suspicion is key to early diagnosis and intervention. The three elements of diagnosis include (i) definition of a clinical phenotype compatible with the diagnosis, (ii) specific serology or radiology, and (iii) histological confirmation if appropriate.

A basic diagnostic evaluation might include blood count and film, inflammatory markers (ESR; CRP), liver function, pancreatic enzymes, ANCA and anti-dsDNA antibodies, rheumatoid factor, complement (C3 and C4), anticardiolipin antibodies, and cryoglobulins. In addition, urine microscopy and a chest X-ray should be undertaken. Immunological investigations might include antistreptolysin O antibody titer (ASOT), ANA, ENA antibodies, ANCA, antiphospholipid antibodies, Immunoglobulins IgG/IgA/IgM/IgE, Complement (C3, C4), and rheumatoid factor.

Infective etiologies might be considered and appropriate investigations might include Tuberculosis screen, viral PCR (e.g., CMV, EBV, enterovirus, adenovirus, VZV, HBV, HCV), Serology for HIV, Rickettsiae, Borrelia burgdorferi, Mycoplasma; Viral serology for Hepatitis B & C, Parvovirus B19). If the differential diagnosis includes autoinflammatory syndromes, then investigations, which might include DNA analysis for MEFV (familial Mediterranean fever), TNFRSF1A (TNF alpha receptor associated periodic fever syndrome, TRAPS), and ADA2 (deficiency of ADA2), should be planned in consultation with an expert rheumatologist.

Further investigations will be directed by the clinical phenotype.

Abdominal ultrasound might allude to bowel wall thickening as might abdominal CT/MRI. CT/MRI might also allude to segmental perfusion defects. Magnetic resonance angiography is not as sensitive as digital subtraction angiography (DSA) in PAN (see below).

Selective visceral DSA is diagnostically very sensitive for systemic PAN and might highlight the site of bleeding if present. Characteristic features of PAN include aneurysms of small- and medium-sized arteries (seen in 40%). Other nonaneurysmal features include arterial cut-off, arterial tapering and stenoses, arterial beading, pruning of the renal arterial tree, and perfusion defects. Overall, arteriographic abnormalities are present in 94% of children with PAN affecting the renal, hepatic, and mesenteric vasculature [18]. Assessment of the GI mucosa by esophagogastroduodenoscopy, video capsule endoscopy, and ileocolonoscopy with biopsy might reveal a plethora of pathologies in vasculitic disorders. Nonspecific colitis is common in childhood systemic vasculitis, and a nonspecific enteritis may be present in SLE [23]. Ulceration may be ischemic or inflammatory in origin. Behcet's ulcers tend to be round, few in number (<5), and focally distributed when compared with Crohn's ulcers, which are more commonly irregular in shape [46]. Ulcers in SLE are typically punched-out. In HSP edema, patchy mucosal redness, erosions, and linear ulceration of the duodenum and ileum are described [46, 47] Purpura similar to that seen in the skin might be seen in the GI mucosa [48]. Mucosal biopsies in Churg–Strauss may demonstrate evidence of eosinophilic GI disease/eosinophilic gastroenteritis.

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Lymphonodular Hyperplasia

33

Tuomo J. Karttunen and Sami Turunen

Introduction

Lymphonodular hyperplasia (LNH) of the gastrointestinal (GI) tract, also known as lymphoid follicular hyperplasia, is characterized by a significant enlargement and often accompanied increase of the numbers of isolated lymphoid aggregates in one or several segments of the GI tract or by a similar alteration of the lymphoid nodules of the Peyer's patches of the distal part of the small intestine. By current criteria, enlargement should be severe enough to make the aggregates elevated and visible by endoscopic assessment or radiology, usually the diameter exceeding 2 or 3 mm in the colon.

LNH is rare in adults and common in children [1–6]. However, true prevalence of LNH is largely unknown as only symptomatic patients have studied, and diagnostic criteria have been variable. In children, LNH has been reported in the duodenum in 16–19% [7, 8], in the ileum in about 50% [9, 10] and in the colon and rectum in about 30–46% [9, 11–13]. In majority of reported series, LNH was the only finding. Clinically, LNH can be asymptomatic or associated with variable set of symptoms including abdominal pain, diarrhoea, bleeding or even intestinal obstruction [3].

LNH is considered a reactive condition of the mucosa-associated lymphoid tissue, with a potential to regress along with disappearance of the inducing stimulus or with maturation of lymphatic system. Inducing factors include microbiological or nutritional luminal factors, immune deficiencies and diseases affecting mucosal integrity. However, exact pathogenic mechanisms are largely unknown, and in many cases, no specific aetiology can be identified. In children, LNH in the colon has long been considered to be an incidental and physiological finding, with spontaneous resolution along with maturation of the mucosal immune system [1, 2, 14]. However, a proportion of paediatric cases seem to associate with non-immunoglobulin E (IgE) food allergies [3, 5].

Definition

LNH has usually been defined as a condition characterized by visible increase of the numbers or size or both of mucosal lymphoid nodules which elevate above the surrounding mucosa. LNH can be seen in any part of the GI tract. Diagnosis is based on endoscopic or radiological appearance, and the confirmation of diagnosis may need exclusion of other diseases causing mucosal elevations, such as various lymphoid neoplasms and reactive and neoplastic polypoid lesions (Table 33.1). This definition does not apply for lesser grades of lymphoid hyperplasia, where either increase of the numbers of lymphoid follicles or their enlargement is of lesser degree so that mucosal elevations are not formed. However, it is likely that there is continuum between such lesser grades of hyperplasia and the endoscopically visible lymphoid nodular hyperplasia [14] fulfilling the current criteria, pointing to the idea that the current criteria are arbitrary, and they do not have a clear pathophysiological basis.

Endoscopic Assessment and Criteria

LNH can be detected by using barium enema [6], but currently, the diagnosis is usually based on endoscopic visualization of typical whitish or mucosa-coloured elevations (Figs. 33.1, 33.2, and 33.3). Image-enhanced endoscopy systems like narrow band imaging or blue laser imaging [15, 16] increase visibility of lymphoid nodules and may need different thresholds for the diagnosis of LNH. In cases where there

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is any uncertainty of the nature of nodules, a complementary histopathological analysis should be used to exclude other disease processes causing endoscopic nodularity or multiple polypoid lesions (Table 33.1). Endoscopic detection of LNH requires active search and is aided by adequate air insufflation during examination as the folds may hide nodularity [5, 11]. LNH can also be detected by capsule endoscopy [17].

Physiologic number and size of isolated lymphoid follicles are variable as they respond dynamically to the signals from diet and luminal flora [18]. In a normal small intestine, the estimated amount is one per 269 villi in the jejunum and one per 28 villi in the ileum [19]. In the proximal colon, the number is 0.02/mm of muscularis mucosae and in the rectosigmoid 0.06/mm [20]. Areal density of lymphoid aggregates in the colorectum was estimated to be 1.1–2.7/cm^2 [21]. Krauss et al. [14] used high-resolution endoscopy objective counting of lymphoid follicles, and they found that in adults, 1–8 lymphoid follicles per endoscopy field of view in the terminal ileum and caecum can be defined as normal. Size of isolated lymphatic follicles has been estimated to be in the range of 0.1–2 mm [19, 20, 22, 23].

Table 33.1 Gastrointestinal diseases to be considered in the endoscopic and histopathological differential diagnosis of LNH

All anatomical areas	
	Giardiasis
	Granulation tissue polyps
	Inflammatory bowel disease
	Lymphangiectasia
	Lymphoma
	Multiple adenomatous or hyperplastic polyps
	Metastatic tumours
	Familial and sporadic gastrointestinal polyposes
	Ganglioneuromatosis
	Neuroendocrine hyperplasia and neoplasia
Stomach	
	Cystic polyps
	Hyperplastic gastritis
Small intestinal mucosa	
	Duodenal Brunner's gland hyperplasia and adenoma
	Duodenal gastric heterotopia
	Groove pancreatitis
Colon and rectal mucosa	
	Solitary rectal ulcer

By definition, diagnosis of LNH needs increase of the size of the lymphoid nodules, but it may be associated with the increase of their numbers. However, there are no consensus criteria for LNH. Considering the size of the nodules, minimum size for a hyperplastic nodule has been suggested to be 2 [11] or 4 mm [24]. The minimum number of nodules has been described as a "cluster", and a cluster has been defined as a group of minimum of 10 nodules [11, 25]. Typically, the size range of polypoid lymphoid nodules is 3–6 mm in diameter.

Terminal ileum contains physiologically elevated lymphoid nodules, that is, Peyer's patches and the diagnosis of LNH is more subjective in this anatomical location. Autopsy studies have indicated that in children the size and number of these patches increases with age [25], and that in adults these are at their highest in the third decade [26]. Kokkonen graded the abundance of ileal lymphoid nodules in four

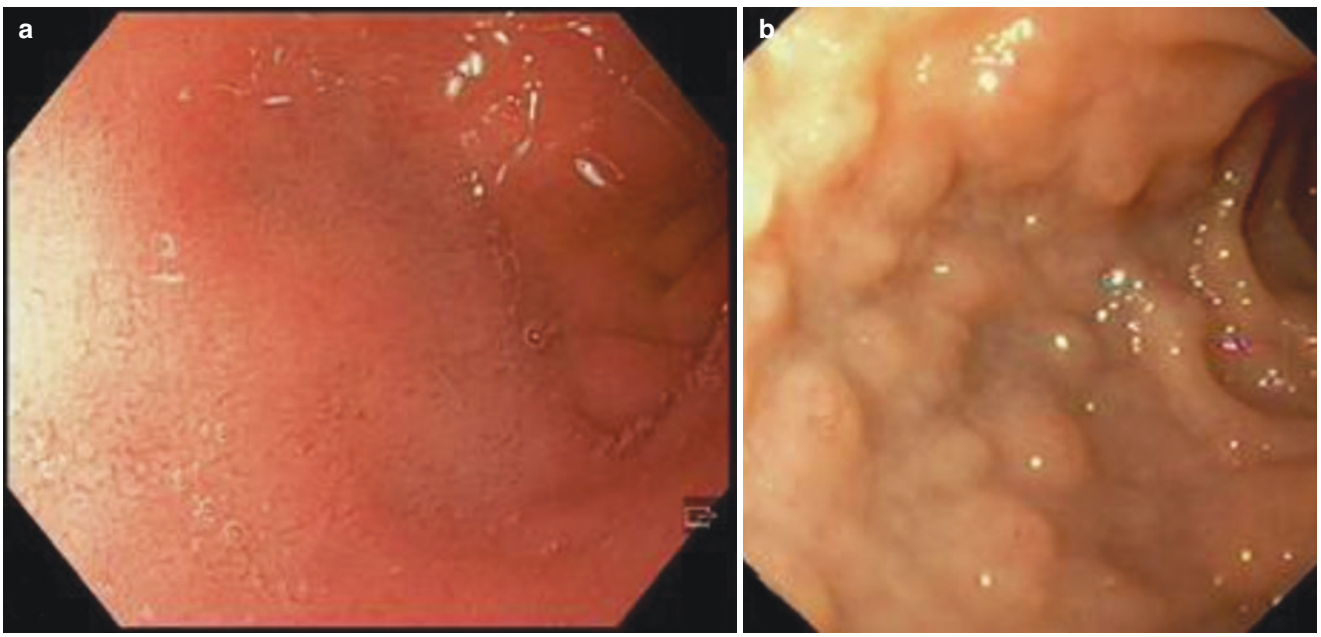

Fig. 33.1 An endoscopic view of the bulb of the duodenum showing normal smooth mucosa (**a**). In lymphonodular hyperplasia, elevations are dispersed along the walls covering the otherwise normal mucosa (**b**)

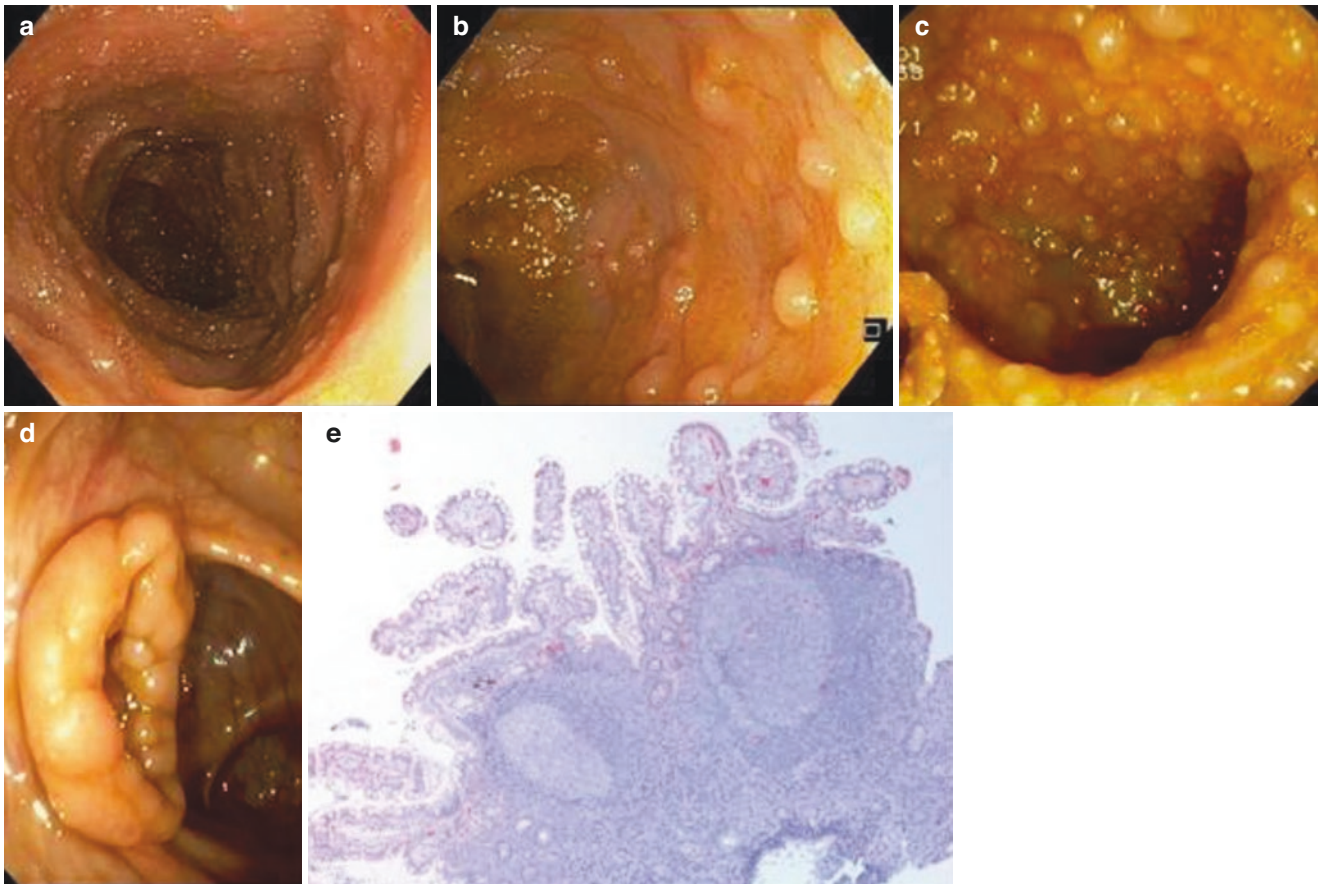

Fig. 33.2 Endoscopic view of the mucosa of a healthy terminal ileum shows usually tiny lymphoid nodules (**a**). In lymphonodular hyperplasia, the nodules are both enlarged and their numbers are increased (**b**) and, in severe cases, may mass on the walls (**c**). In some cases, enlarged lymphoid nodules may extend to the region of ileocaecal valve and be visible even from the caecal side (**d**). The biopsy taken from the terminal ileum with lymphonodular hyperplasia may be filled with lymphoid tissue as large nodules. However, normal long villi can be detected between the nodules (**e**)

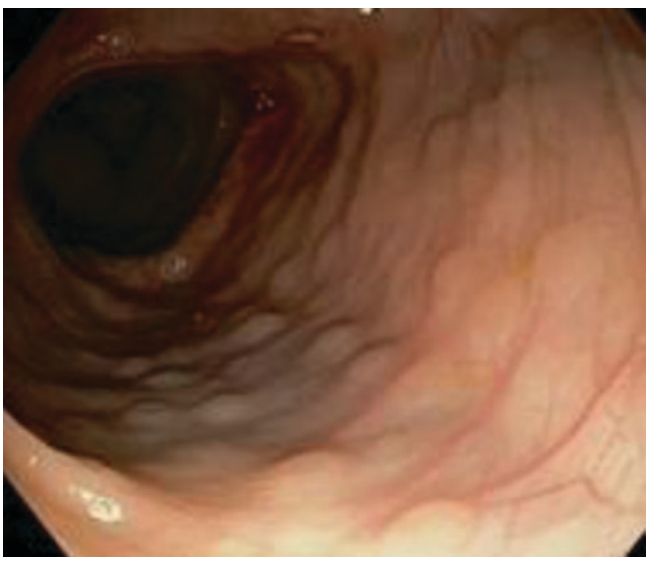

Fig. 33.3 Endoscopic view of colon showing lymphonodular hyperplasia

grades: 0, no lymphoid nodules; 1, mild, lymphoid nodules dispersed on the walls; 2, moderate, lymphoid nodules filling the walls; 3, severe, terminal ileum massed with lymphoid tissue, valve protruding [5] (Fig. 32.2). Counts of lymphoid nodules per visual field of a high-resolution endoscopy in adults in the terminal ileum and caecum indicated that 9–50 lymphoid nodules per field can be defined as lymphoid hyperplasia, and that more than 50 lymphoid nodules per field can be defined as pathological lymphoid aggregates [14].

According to current criteria, increase of the numbers or size of lymphoid nodules less than 2 mm and without endoscopic evidence of nodularity is not diagnostic for LNH. However, as the development of LNH is gradual, even the lesser grades with only numerical increase or increase of the size of nodule without formation of any elevation could have significance. It is obvious that we need more data on the pathophysiology and clinical significance of such lesser grades of lymphoid hyperplasia.

Anatomical Distribution of LNH

LNH may affect any part of the GI tract, and simultaneous manifestation in several parts is not uncommon [5, 9]. Distribution of lesions may provide information of aetiology of LNH (Table 33.2). LNH related with infectious aetiologies tends to be localized. Similarly, LNH related with motility disorders is mostly localized to or close to the affected segment. In contrast, LNH related with immunodeficiency is often diffusely present in the intestinal mucosa. LNH associated with food allergy is often multifocal, but in the duodenum, the bulb is the most commonly affected location [7].

Histological Assessment of LNH

Biopsy specimens from an LNH lesion typically show a single lymphoid follicle or a group of follicles, usually with germinal centres (Fig. 33.2). Such structures are normal components of mucosa, and their occurrence by such is therefore not diagnostic for LNH. Germinal centres are rarely seen in normal lymphoid follicles of healthy gastric [27, 28] or duodenal mucosa. In LNH, a germinal centre is usually present in the follicle and the follicle is enlarged. There is physiological variation in the size of follicles and no criteria regarding a threshold size for histological detection of LNH have been determined. Furthermore, how the tissue sections available represent the largest diameter of the structure makes determination of size difficult. Elevation in comparison with the height of adjacent mucosa is sometimes visible in sections, and suggests LNH. However, such criteria are not so well usable for the second type of the isolated lymphoid follicles of the colon located predominantly in the submucosa [29].

Table 33.2 Aetiology or associated condition of lymphonodular hyperplasia in different parts of the gastrointestinal tract

Part of gastrointestinal tract	Aetiology/associated condition
Stomach	*Helicobacter pylori* infection
Duodenum	Food allergy, non-IgE IBD
	Juvenile idiopathic arthritis
	Immune deficiency (IgA, CVID, APDS, HIV)
	Giardiasis
Ileum	Food allergy, non-IgE Infection (*Salmonella*, *Yersinia*, adenovirus) IBD
Colon	Food allergy, non-IgE
	IBD
	Immunodeficiency (IgA, CVID, APDS, HIV) Irritable bowel syndrome
	Infectious enteritis Hirschsprung's disease Constipation (idiopathic and food allergy associated)
Rectum	IBD
	Chlamydia infection

IgA immunoglobulin A, *CVID* common variable immune deficiency, *IBD* inflammatory bowel disease

In LNH alike reactive germinal centres, tangible body macrophages and a network of dendritic cells are present [30]. Germinal centres are surrounded by a mantle zone containing T and B lymphocytes [29]. Inter-follicular tissue may have some features of the para-cortical zone of lymph nodes, such as high endothelial venules, however, expressing here the mucosal addressin MadCam1 [29, 30].

In the intestine, epithelium overlying lymphoid follicles contains enterocytes and a specialized epithelial cell type M cells [29], showing characteristic microfolds. M cells contain pockets with intra-epithelial lymphocytes. Functionally, M cells act as transporters of luminal antigens to be processed by the cells of lymphatic follicle.

As additional histopathological features in some cases of LNH, observed in children with delayed type of food allergy, the intestinal mucosa may show increase of intra-epithelial lymphocytes including gamma–delta-expressing lymphocytes and cytotoxic lymphocytes [8, 10, 31]. Similarly, the numbers of intra-epithelial eosinophilic leukocytes may be increased in LNH cases associated with food allergy [11]. In cases where LNH associates with specific disease or condition, these may present with characteristic features, such as crypt distortion and diffuse increase of inflammatory cells in IBD-associated cases, and characteristic decrease or absence of plasma cells in common variable immune deficiency [32].

Histopathological differential diagnosis of LNH mainly involves malignant lymphomas of mucosa. They are very rare in children, but the risk is increased in children with immune deficiency or solid-organ transplantation.

Pathophysiology of LNH

GI mucosal lymphatic tissue consists of inductive and effector compartments [33–35]. Luminal antigens are processed, and specific immune responses are generated in the inductive compartment composed of Peyer's patches in the ileum and isolated lymphoid follicles in other parts of the GI tract. Isolated lymphoid follicles are aggregates of T and B lymphocytes and located in either mucosa or submucosa, latter extending to mucosa [19, 29]. The effector compartment is the diffuse mucosal lymphatic infiltrate which is composed of immunoglobulin-producing B cells and effector T cells [29, 33–35].

Luminal commensal and pathogenic flora induces production of IgA response within Peyer's patches and isolated lymphoid follicles [33–35], accompanied with IgM, IgG and IgD responses [35]. At least in mice, such responses involve formation of new lymphoid follicles [18]. Both development and neogenesis are composed of interaction of sub-epithelial stromal cells, epithelial stem cells and lymphoid cells [18, 34, 35]. Germinal centres formed are the places where *IgA* genes of the B lymphocytes mutate and relevant clones are selected. There is evidence that T-independent pathways can give rise to intestinal IgA-secreting plasma cells in isolated lymphatic follicles and diffuse lamina propria, while Peyer's patches can support both T-dependent and T-independent modes of IgA induction [18, 34, 35].

Since the secreted IgA is induced by luminal microbes and other antigens and also participates in the control of the amount of luminal microbial flora, the LNH might be viewed as a sign of IgA dysfunction, where host–microbiome balance is not reached within normal structural limits of lymphoid tissue [18, 19, 35]. Accordingly, also LNH in association with immunodeficiency might be explained as compensatory mechanism to gain the balance. Similarly, increase of the numbers of lymphoid follicles in the colon of patients with infectious colitis [14], or gastric LNH in association with *Helicobacter pylori* infection [7, 27, 28, 36], may represent similar attempt to control the infection by increased production of IgA and suggests that both increase of numbers of lymphoid follicles and their size may be involved. Children with LNH may show more abundant bacterial flora in the ileum than the control children [37], supporting the role of microbial factors in the pathogenesis of LNH. Although not documented, factors modifying access of microbes and other luminal antigens to the mucosa, such as innate immunity responses, mucin and abnormal epithelial permeability [38], might contribute to enhanced humoral immune response involving development of LNH.

Mechanisms linking non-IgE food allergy and LNH [3] are not clear. There is only limited experimental evidence for the role of dietary antigens in mucosal germinal centre response [39]. Association of history of IgE-mediated milk allergy in infancy and increase of anti-allergen IgA levels and LNH [40] supports the idea that LNH, by providing IgA anti-allergen response, might represent an attempt to tolerance [41].

Mechanisms of segmental localization of LNH with some preference to locate close to sphincter-like structures (distal to pylorus; proximal to ileocaecal valve), and association with motoric dysfunction (obstipation and Hirschsprung's disease), are speculative. However, stagnation of intestinal contents related with these anatomical structures or neuromuscular dysfunction might lead the mucosa to be exposed longer to luminal antigens, and thereby increase the local antigen load.

The role of alteration of immune regulatory mechanisms in pathogenesis of LNH is not clear. Bellanti et al. [42] found evidence of deficient T helper cell (Th)1 function in peripheral blood of children with food allergy associated with LNH. In children with delayed type of milk allergy, where majority of patients show LNH, ileal mucosa showed increase of interleukin 6 (IL-6) messenger (mRNA) [43], and duodenal mucosa showed increase of interferon (IFN) gamma mRNA levels [44], increased number of cells expressing IFN gamma [45] and increased IFN gamma, IL-4 and IL-10 secretion [44], possibly indicating imbalance of pro- and anti-inflammatory cytokines.

LNH and Associated Conditions: Food Allergy

Of the conditions associated with LNH (Table 33.2) food allergy is the most frequent. In children, studies performed in four different populations, in Finland [7–9, 46], Italy [11], the USA [42] and Turkey [47], have shown that LNH in duodenal or ileo-colonic mucosa associates with food allergy of the non-IgE type, the most common allergen being milk. Similar association between allergy and ileal and colon LNH has been observed in adults [14, 48]. In contrast, Troncone [49] and Lucarelli [13] found evidence for food allergy in only a minority of symptomatic children with LNH. A review by Mansueto [3] concluded that LNH is present in 49% (range 32–67) of children with food allergy, and conversely, about 66% (range 42–90) of children with LNH have food allergy. In these studies, food allergy was diagnosed based on food challenge-elimination tests, but not all studies were based on state-of-the-art blinded tests [3]. Skin prick tests and serum IgE-based tests were mostly negative. Response time in food challenge test was long – at least several days. These findings indicate that food hypersensitivity associated with LNH is of non-IgE type and characterized by a slow response times of usually several days.

Symptomatic and endoscopical response to elimination diet in delayed type of food allergy associated with LNH has been documented in both adults and children [11, 48]. However, in some studies, the effects of food exclusion did not differ from sell-limiting clinical course with symptomatic treatment [13].

LNH in Other Diseases and Conditions

Chronic mucosal infections (Table 33.2) manifesting in some cases with local LNH include *H. pylori* gastritis [4, 7, 36]. Some enteric bacterial infections may lead to ileal LNH. Giardiasis causes sometimes small intestinal LNH, usually in background of humoral immune deficiency [50].

Chlamydia infection causes some cases of rectal LNH [51]. In general, intestinal bacterial infections increase the number of endoscopically detectable lymphoid follicles [18] and are associated with LNH [16]. LNH may associate with human immunodeficiency virus (HIV) infection [52].

LNH extending through the small intestine associates with genetic immune disorders such as common variable immune deficiency (CVID), where LNH develops in about 20% of cases [53]. Selective IgA deficiency is similarly associated with LNH, nodules containing IgM-expressing cells instead of IgA expression [50]. LNH is one intestinal manifestations of activated PI3Kδ Syndrome (APDS), a newly described form of primary immunodeficiency [54]. In PTEN hamartoma tumour syndrome, where PTEN mutations lead to autoimmune diseases, about 43% of patients have LNH in the intestine [55]. Ileo-colonic LNH associates with familial Mediterranean fever [47].

LNH has been reported in juvenile idiopathic arthritis and connective tissue diseases in the duodenum, ileum and colon [56].

LNH has rarely been described in inflammatory bowel disease, including both ulcerative colitis and Crohn's disease [5, 9, 11]. However, no association with mutations of nucleotide-binding oligomerization domain-containing protein 2/caspase recruitment domain-containing protein 15 gene (NOD2/CARD15) has been detected in LNH [57]. Lymphoid hyperplasia has been found in Hirschsprung's disease in the aganglionic segment [58].

In adults, colonic LNH has been reported in about 31% of patients with irritable colon syndrome [16].

A rare form of segmental lymphoid hyperplasia is located in the distal rectum of adults and children and has been referred to a rectal tonsil [59]. Clinically, rectal tonsil is often associated with rectal bleeding and abdominal pain, but pathophysiology is unknown. However, similar constellation may be caused by a local chlamydia infection [51].

Lymphoid follicular proctitis is a rather poorly defined condition, where rectal mucosal nodularity is related with formation of lymphoid follicles and inter-follicular chronic inflammation. Symptoms are composed of mucoid stools and haematochezia, and occasional response to sulfasalazine treatment suggests that some cases represent a variant of inflammatory bowel disease (IBD) [60].

There are reports suggesting ileal or ileo-colonic LNH to be a common abnormality in children with autistic spectrum disorders, but these findings are highly controversial [3].

Symptoms of LNH

Symptoms in children with LNH are listed in Table 33.3. It is not clear what symptoms can be attributed specifically to LNH as there is no consistent pattern, and some patients have no symptoms. It is obvious that conditions underlying or associating with LNH, such as immune deficiency, constipation or IBD, contribute to symptoms. In cases with LNH as a sole finding, LNH could just be a marker of mucosal immunological activation and not mechanistically contributing to symptoms. In this context, it is of interest that symptoms associated with LNH overlap with those in irritable bowel syndrome, and these conditions share occurrence of signs of overall mucosal immune activation, such as increased intraepithelial lymphocytes in both [7, 61].

Table 33.3 Clinical symptoms commonly reported in paediatric patients examined with gastroduodenoscopy and/or colonoscopy and having nodular lymphoid hyperplasia as an endoscopic finding [2, 3, 5, 11, 42, 46]

Recurrent abdominal pain
Blood in stools
Diarrhoea
Constipation
Anaemia
Nausea/vomiting
Growth retardation or weight loss

Some symptoms may be more specifically caused by LNH. Severe LNH in the terminal ileum can lead to invagination (intussusception). Common association of bloody stools and LNH [11, 12, 48] can be mechanistically explained by sensitivity for mechanical damage associated with any elevated mucosal lesion. Kaplan et al. [12] found that LNH in the colon was commonly associated with friability and ulceration. Observations in Crohn's disease have suggested that M cells overlying the lymphoid follicles form the most vulnerable population of surface epithelium [62], but whether M cells in LNH with other conditions are specifically vulnerable is not known.

Treatment and Prognosis

LNH is the manifestation of underlying immune response and not a specific disease. The initiating factors should be searched for (Table 33.2), and treatment targeting on the aetiology should be considered. In children with irritable colon-like symptoms and idiopathic LNH as a sole finding, budesonide has been used, but no formal trials are available [5].

No systematic and long-term follow-up studies of the development of LNH with or without treatment are available. Partial regression of LNH has been detected in some follow-up endoscopy studies within a year in cases associated with food allergy and treated with elimination diet [11]. In other studies [5], treatment with elimination diet did not induce any change in the LNH grade, although the number of intraepithelial lymphocytes diminished towards normal levels. Lucarelli et al. [13] reported that majority of symptomatic children with ileo-colonic LNH, most without food allergy,

improved clinically in 2–12 months with symptomatic treatment.

There is only scanty information of long-term prognosis of LNH. Risk of intestinal lymphoma is increased in LNH in association with immunodeficiency. In adults, risk is more significant, and some cases even without immunodeficiency have been reported [4]. Colon et al. [2] did not detect any long-term consequences in their unselected cases with LNH. Incidence rates of LNH in adults seem to be much less than children [4], and the overall prevalence of LNH seems to decrease with age, supporting the idea that major proportion of childhood cases not associated with immune deficiency are resolved before adulthood.

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Acute Pancreatitis

34

Jonathan Wong, Praveen S. Goday, and Steven L. Werlin

Anatomy and Physiology

The pancreas is a large, J-shaped, flattened gland located in the upper left abdomen that secretes digestive enzymes into the duodenum and produces several important hormones as part of the endocrine system. In adults, it is about 15–20 cm long, lies inferior to the stomach, and is surrounded by the small intestine, spleen, gallbladder, and liver [1, 2]. The pancreas is composed of four sections: head, neck, body, and tail. The uncinate process emerges from the head of the pancreas and lies deep to the superior mesenteric artery and vein, which run behind the neck of the pancreas [3].

The pancreas is both an exocrine and endocrine gland. The exocrine pancreas is made up of acinar and ductal cells that produce and transport digestive enzymes, such as amylase, lipase, and trypsin through a system of small ducts that lead to the pancreatic duct, which runs the length of the pancreas and then combines with the common bile duct carrying bile from the gallbladder proximal to the ampulla of Vater (Fig. 34.1a) [1]. Pancreatic enzymes and bile are then secreted into the small intestine and aid in the digestion of carbohydrates, fats, and proteins. The endocrine cells of the pancreas, located in the islets of Langerhans, produce two main hormones, insulin and glucagon, which together control glucose metabolism. The endocrine cells also produce other hormones including somatostatin and pancreatic polypeptide [4–6].

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Pathophysiology

Pathological trypsinogen activation has long been considered the hallmark of AP. Following an initial insult, such as ductal disruption or obstruction, cathepsin B activates trypsinogen to trypsin within the acinar cell. Trypsin then activates other pancreatic proenzymes, leading to autodigestion, further enzyme activation, and release of active proteases. Lysosomal hydrolases colocalize with pancreatic proenzymes within the acinar cell. Pancreastasis (similar in concept to cholestasis) with continued synthesis of enzymes occurs. Lecithin is activated by phospholipase A2 into the toxic lysolecithin. Prophospholipase is unstable and can be activated by minute quantities of trypsin [7, 8]. This leads to cell death and AP. After the insult, cytokines and other proinflammatory mediators are released. Most animal models support this mechanism.

The healthy pancreas is protected from autodigestion by pancreatic proteases that are synthesized as inactive proenzymes; which are then segregated into secretory zymogen granules at pH 6.2, by low calcium concentration, which minimizes trypsin activity. Protease inhibitors are present both in the cytoplasm and zymogen granules [5–8]. Enzymes are secreted directly into the ducts, lessening exposure of the cytoplasmic contents.

In animal models, intracellular activation of trypsinogen to trypsin plays a role only in early acinar injury. For example, in a knockout mouse model of pancreatitis in mice lacking the trypsinogen 7 gene, pancreatitis still develops even though they are unable to activate trypsinogen [8, 9]. In this model, NFkB is activated early in the course and appears to be the primary driver of the proinflammatory response. Recent studies have shown perturbation of mitochondrial permeability and function early in the evolution of experimental pancreatitis [10]. In this model, alcohol causes a collapse of the electrical gradient across the mitochondrial permeability transition pore, leading to depletion of ATP and acinar cell necrosis.

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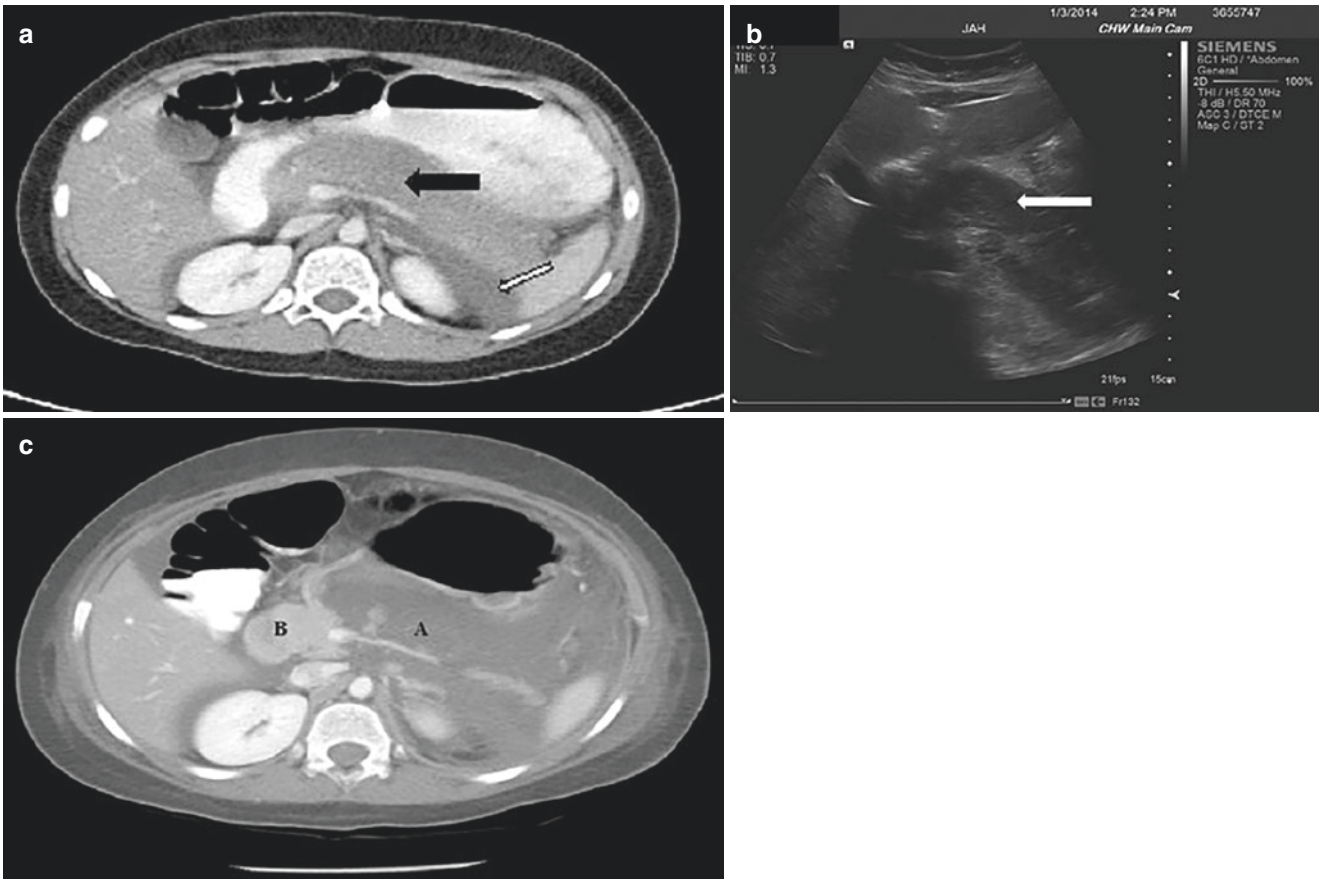

Fig. 34.1 MRCP changes in chronic pancreatitis. (**a**) Early acute pancreatitis. Note edematous gland (black arrow) with a small amount of fluid (small arrow). No visible ducts or calcifications. (**b**) Ultrasound of the same patient showing a hypoechoic and edematous pancreas (arrow). (**c**) Late acute pancreatitis in the same patient CT scan demonstrates necrotic nonenhancing distal pancreas (**A**). The pancreatic head is relatively normal (**B**). There is a large amount of peripancreatic fluid (**C**). (Courtesy of Dr. David Gregg)

These observations may be related to abnormal cell signaling as a result of pathological elevation of Ca^{2+} in acinar cells. Abnormal sustained release of Ca^{2+} (as can be seen in excessive levels of cholecystokinin, alcohol, or bile acids) can cause mitochondrial permeability transition pores to open, resulting in loss of membrane potential and subsequent impaired production of ATP. Pathologically elevated Ca^{2+} concentrations have also been implicated in premature trypsinogen activation, impaired autophagy, and activation of the NFkB pathway [11].

Time-course analysis has shown that a burst of electron transfer reactions is associated with the disease-initiation [12]. For example, in the experimental model of mild AP produced by excessive stimulation with caerulein, a CCK analog, the spark from reactive oxygen species can be seen by chemiluminescence within 5 minutes. Simultaneously, there is a huge increase in stress-activated protein kinase. Within 10 minutes, there is an increase in amylase in the venous outflow of the pancreas. Similarly, in endoscopic retrograde cholangiopancreatography (ERCP)-induced acute pancreatitis, analysis of peripheral blood by electron spin resonance spectroscopy identified the burst of reactive oxygen species by the end of the clinical procedure, followed by steep increases in serum levels of amylase, lipase, and trypsinogen.

Histopathologically, interstitial edema appears early. Later, as the episode of pancreatitis progresses, localized and confluent necrosis, blood vessel disruption leading to hemorrhage, and an inflammatory response in the peritoneum can develop. In mild pancreatitis, there is interstitial edema and an inflammatory infiltrate is found. There is no organ dysfunction. In severe pancreatitis, the inflammation is extensive and parenchymal necrosis is present. Multiorgan failure accompanies the inflammation. Following an episode of AP, all histological abnormalities resolve. The factors that determine the severity of an episode of AP are unknown; furthermore, scoring systems utilized in adult patients (Ranson, Glasgow) are not applicable in children [13].

Epidemiology

In adults, AP is one of the most common diseases of the gastrointestinal tract and remains a serious disease. In the United States, AP is the most common gastroenterology discharge diagnosis accounting for more than 553,000 hospital discharges, 881,000 ambulatory visits, and 3413 deaths a year [14, 15]. A recent systematic review demonstrated a global pooled incidence of AP of 34 cases per 100,000 general population per year, with equal incidence in men and women [16].

In pediatrics, it is estimated that approximately 10 new cases per 100,000 children occur annually. In adults, AP is ranked 15th among causes of death from gastrointestinal and liver diseases in adults [17]. In the last 20 years, studies have shown an increase in the annual incidence of AP [18]. The case fatality rate for AP has decreased over time, while the overall population mortality rate has remained unchanged. In adults, mortality from AP is approximately 3% for interstitial pancreatitis and 15% for necrotizing pancreatitis [14]. A recent study of over 2000 pediatric cases of AP treated in an ICU setting found mortality of primary and secondary pancreatitis to be 0.3% and 6.8%, respectively. These data suggest that AP in otherwise well children is unlikely to be fatal but that when AP develops in the setting of other acute or chronic illnesses, outcome may be dictated by that primary illness [19].

Etiology

In adults, 85% of episodes of AP are due to alcohol and cholelithiasis. In contrast, in children, the most common etiologies of AP are: blunt abdominal injuries, biliary stones or microlithiasis (sludging), drug toxicity, and multisystem diseases such as the hemolytic uremic syndrome and inflammatory bowel disease [13, 20, 21]. Other cases follow solid organ and stem cell transplantation or are due to infections, anatomic anomalies, metabolic disorders, and mutations in susceptibility genes. Only 10–20% of cases are now considered idiopathic.

Trauma typically due to bicycle handlebar injuries, automobile accidents, and sports injuries is the cause of about 10–40% of episodes of AP [13, 20, 21] (Table 34.1). Other traumas include ERCP, nonaccidental injury, surgical injury, and total body casting. Because the pancreas is retroperitoneal and lies across the spine, ductal rupture is not uncommon. Diagnosis may be delayed. Most patients with abdominal trauma who are seen in the emergency room get a CT scan; injuries may now be detected earlier than before. Following trauma, unsuspected ductal damage can lead to strictures, pseudocyst formation, and chronic obstruction.

Table 34.1 Traumatic causes of acute pancreatitis

Blunt injury
Nonaccidental injury
ERCP
Head trauma
Surgical trauma
Total body casting

Table 34.2 Biliary tract causes of pancreatitis

Ampullary disease
Ascariasis
Biliary tract malformations
Cholelithiasis, microlithiasis, and choledocholithiasis
Duplication cyst
Endoscopic retrograde cholangiopancreatography (ERCP) complication
Pancreas divisum
Pancreatic ductal abnormalities
Pancreaticobiliary maljunction
Choledochal cyst
Choledochocele
Postoperative
Sphincter of Oddi dysfunction
Tumor

Biliary pancreatitis Biliary obstruction due to lithiasis, sludge, anatomic abnormalities, or ERCP is the etiology in 5–20% of pancreatitis in children (Table 34.2). Anatomic causes of biliary obstruction such as pancreaticobiliary maljunction (PBM) and pancreas divisum are increasing recognized [22–24]. PBM and congenital dilatation of the biliary tract are more common in Japanese patients than in western patients. Controversy persists over whether pancreas divisum alone is a cause of AP. Recent studies have not supported either a clear association or a definitive line of treatment for pancreatic disease in the setting of pancreas divisum [23, 24]. Risk factors for biliary pancreatitis in children include obesity and Hispanic ethnicity [25].

Drugs and toxins Drugs and toxins account for 10–20% of children with AP (Table 34.3). In children, valproic acid, L-asparaginase, 6-mercaptopurine, and azathioprine are the most common causes of drug-induced pancreatitis [26]. Azathioprine has on occasion been successfully reintroduced in patients with presumed thiopurine-induced pancreatitis [27].

Infectious Agents Since many patients with idiopathic pancreatitis have a viral-like prodrome, it is difficult to determine the true incidence of infection-associated AP (Table 34.4) [13, 20, 21]. Case series have found that 2–10% of children with AP have a viral cause. This may be an under-

estimate, because many patients with idiopathic pancreatitis may well have had undiagnosed infections. A wide variety of infections have been associated with AP particularly hepatitis A, mumps, and EBV.

Genetics Mutations in an increasing number of genes have been shown to cause pancreatitis, the most common being mutations in the PRSS1, CFTR, and SPINK1 genes. Genetic causes have been reported in 1–14% of cases [28, 29] (Table 34.5). The incidence may well be higher, since it is uncommon to check for genetic etiologies during the first episode of pancreatitis. PRSS1 is the gene that causes *hereditary pancreatitis*. Atypical cystic fibrosis in patients with at least one mild mutation and pancreatic sufficiency are at risk for pancreatitis. At the time of a first episode of AP, patients with mutations in these genes will have courses indistinguishable from patients with pancreatitis from other causes. Unless there is a family history of pancreatitis or cystic fibrosis or there are signs of chronicity on imaging studies, genetic testing is not indicated following a single episode of AP.

Table 34.3 Drugs and toxins

Acetaminophen
Alcohol
[a]L-Asparaginase
[a]Azathioprine
Carbamazepine
Cimetidine
Corticosteroids
Didanosine
Enalapril
Erythromycin
Estrogen
Furosemide
Isoniazid
Lamuvidine
Lisinopril
[a]6-Mercaptopurine
Methyldopa
Metronidazole
Octreotide
Opiates
Organophosphate poisoning
Pentamidine
Phenformin
Retrovirals: zalcitabine, didanosine, tenofovir
Simvastatin
Sulfonamides
Sulindac
Tetracycline
Thiazides
[a]Valproic acid
Venom (spider, scorpion, Gila monster lizard)
Vincristine

[a]Most common in children

Systemic and autoimmune disease Pancreatitis is well known to be associated with a number of systemic diseases, particularly hemolytic uremic syndrome, sepsis, and shock [13, 20, 21] (Table 34.6). The incidence of AP associated with these conditions ranges from 5% to 35% in published

Table 34.4 Infectious causes of pancreatitis

Ascariasis
Coxsackie B virus
[a]Epstein-Barr virus
[a]Hepatitis A
Hepatitis B
Influenza A, B
Leptospirosis
Malaria
Measles
[a]Mumps
Mycoplasma
Rubella
Rubeola
Reye syndrome: varicella, influenza B
Septic shock

[a]Most common in children

Table 34.5 Genetic causes of pancreatitis

PRSS1: cationic trypsinogen
CTRC: Chymotrypsin C gene
CFTR: Cystic fibrosis gene
SPINK 1: Trypsin inhibitor gene
CPA2: Carboxypeptidase A2
CASR Calcium-sensing receptor
CLDN2: Claudin 2

Table 34.6 Systemic and autoimmune diseases

Autoimmune pancreatitis
Burns
Collagen vascular diseases
Crohn's disease
Hypercalcemia
Diabetic ketoacidosis
Hemochromatosis
Hemolytic uremic syndrome
Hyperlipidemia: type I, IV, V
Hyperparathyroidism/Hypercalcemia
Kawasaki disease
Malignancy
Malnutrition
Metabolic diseases: organic acidemia
Peptic ulcer
Polyarteritis nodosa
Renal failure
Solid organ transplant
Systemic lupus erythematosus
Transplantation: stem cell, solid organ
Vasculitis
Hypothermia

series. These cases typically have a course similar to that of pancreatitis from other causes. Autoimmune pancreatitis is rare in children; a 2017 study reviewing case reports over the preceding two decades along with data from two recently established databases (INSPPIRE, CUSL) yielded only 48 cases [30].

Idiopathic In published series, no etiology was found in 12–38% of children with AP [13, 20, 21]. As new etiologies have been found and as workup has become more extensive, newer series have lower rates of idiopathic AP.

Diagnosis

Criteria for the diagnosis of pancreatitis are defined as 2 of 3 of the following: abdominal pain, serum amylase, and/or lipase activity at least 3 times greater than the upper limit of normal and imaging findings characteristic of, or compatible with, AP [13].

Serum lipase is the test of choice for AP, as it is more specific than amylase for acute inflammatory pancreatic disease and should be determined when pancreatitis is suspected. Serum lipase remains elevated longer than amylase after disease presentation. The serum lipase rises by 4–8 hours, peaks at 24–48 hours, and remains elevated 8–14 days, longer than serum amylase. Serum lipase may also be elevated in nonpancreatic diseases (Table 34.7). Diabetic patients appear to have a higher median lipase compared with nondiabetic patients, so an upper limit of normal greater than 3–5 times may be needed in diabetic patients [31]. The clinical condition of the patient must be considered when evaluating amylase and lipase elevations.

In AP, serum amylase usually rises within a few hours after the onset of symptoms and returns to normal within 3–5 days. Serum amylase may remain normal in up to 20% of patients, especially in alcohol-induced AP and hypertriglyceridemia [32]. A variety of conditions may also cause hyperamylasemia without pancreatitis, such as macroamylasemia (Table 34.7).

Providers interpreting amylase and lipase levels in a chronological context should note that as the kidneys excrete these enzymes, nonpancreatic-based elevations may be seen in patients with renal injury or disease [13].

Other laboratory abnormalities that may be present in AP include hemoconcentration, manifested by high hemoglobin and BUN, coagulopathy, leukocytosis, hyperglycemia, glucosuria, and hypocalcemia. Elevated γ-glutamyl transpeptidase and hyperbilirubinemia suggest the diagnosis of cholelithiasis or choledocholithiasis [13, 14].

X-rays of the chest and abdomen may demonstrate nonspecific findings such as atelectasis, basilar infiltrates, elevation of the hemidiaphragm, left- (rarely right-) sided pleural effusions, pericardial effusion, and pulmonary edema. Abdominal x-rays might demonstrate a sentinel loop of intestine, dilation of the transverse colon (cutoff sign), ileus, pancreatic calcification (if recurrent), blurring of the left psoas margin, a pseudocyst, ascites, and peripancreatic extraluminal gas bubbles [14, 33].

Table 34.7 Nonpancreatitis causes of elevated serum pancreatic enzymes

Serum lipase	Serum amylase
Acute cholecystitis	Acidosis
Bowel obstruction	Alcoholism
Celiac disease	Appendicitis
Diabetic ketoacidosis	Bowel obstruction/infarction
Drugs	Celiac disease
Duodenal ulceration	Cerebral trauma
Human Immunodeficiency Virus	Cholecystitis
Idiopathic	Cystic fibrosis
Macrolipasemia	Drugs
Pancreatic calculus	Human Immunodeficiency Virus
Pancreatic tumors	Idiopathic
Renal failure	Liver disease
Trauma (including post-ERCP)	Lymphoma
	Macroamylasemia
	Myocardial infarction
	Pancreatic tumors
	Pelvic inflammatory disease
	Peptic ulcers
	Pregnancy
	Renal failure
	Rheumatoid arthritis
	Trauma
	Ulcerative colitis

Transabdominal ultrasound should be performed on all patients with AP, when gallstones are suspected. Abdominal imaging is useful to confirm the diagnosis of AP, but the routine use of computed tomography (CT) is not recommended during the first 3 days, because it has low yield and rarely alters clinical management [14, 13, 33]. Abnormal imaging findings (such as signs of necrosis) may not develop until 72 hours after symptom onset. Given this, obtaining a CT scan within 72 hours of symptom onset is discouraged by present guidelines [11]. However, if a patient is not improving after 1 week, CT or magnetic resonance imaging (MRI) of the pancreas is recommended to assess for local complications (Fig. 34.1b). CT and/or MRI of the pancreas are also recommended in patients in whom the diagnosis is unclear. CT has over 90% sensitivity and specificity for the diagnosis of AP. Ultrasonography is more sensitive than CT scanning for the diagnosis of biliary stones but can be limited in evaluation of the pancreas itself due to interfering structures [13, 14].

Magnetic resonance cholangiopancreatography (MRCP) and ERCP are essential in the investigation of known or

suspected pancreatic duct disruption, recurrent and nonresolving pancreatitis, and disease associated with gallbladder pathology but have a lesser role in AP [13, 34]. Although there are limited pediatric data, endoscopic ultrasonography has increasingly been shown to be helpful in the drainage of fluid collections in children [35].

Clinical Presentation

Although there have been a number of attempts to develop a scoring system, there is no validated scoring system for children with AP [36, 37]. A retrospective report of 211 patients suggested that a serum lipase >7 times the upper limit of normal within 24 hours of admission predicted a severe course [38]. This however was not been confirmed [13]. Thus, reliable prediction of the course of an episode of AP is currently not possible. Several new classification systems have been proposed for use in adults. None have been tested in children.

Most episodes of AP in children are mild and self-limiting, defined by the absence of organ failure and/or pancreatic necrosis (local complications) [39]. These patients resolve their symptoms rapidly, are usually discharged within 1 week, and mortality is rare [13, 14].

Pediatric AP is classified as mild, moderate, and severe.

Mild Acute Pancreatitis

Cases of mild AP typically resolve within 1 week without local or systemic involvement [40]. The patient with AP has abdominal pain, which may be severe. Some patients have persistent vomiting, and possibly fever. The pain is epigastric or in either upper quadrant. The child often lies with hips and knees flexed lying on the side. The abdomen may be distended and tender, and a mass may be palpable. The patient usually appears extremely uncomfortable, irritable, and may look ill or toxic. The pain can increase in intensity for 24–48 hours, during which time vomiting may increase and the patient might require hospitalization for dehydration and may need fluid and electrolyte therapy. Pain described as dull, colicky, or located in the lower abdomen is not consistent with AP and suggests other etiologies. The prognosis for complete recovery in the acute uncomplicated case is excellent [13, 14, 39].

Moderate Acute Pancreatitis

Patients with moderately severe AP may have transient organ failure that resolves in less than 48 hours, local complications, exacerbation of comorbid disease, and/or systemic complications. The morbidity and mortality are higher compared to that of mild AP. However, the mortality is considerably less than that of severe AP [14, 19, 41]. Depending on the complications, patients may require prolonged hospitalization because of local or systemic complications.

Severe Acute Pancreatitis

Severe AP is defined as AP complicated by persistent organ failure that persists beyond 48 hours and/or death and occurs in 15–20% of patients [14, 19, 41]. Patients with severe AP usually have one or more local and/or systemic complications.

Severe AP is less common in children than in adults [37]. In this life-threatening condition, the patient is acutely ill with severe nausea, vomiting, and abdominal pain [14, 33, 41]. A bluish discoloration may be seen around the umbilicus (Cullen sign) or in the flanks (Grey Turner sign) [42, 43]. Pancreatic necrosis may develop between the second and fifth weeks of illness. Infected necrosis may develop during the second week and a pancreatic abscess as late as the fifth week of illness. Infection is suspected when there is persistent pain and fever.

Mortality is related to the systemic inflammatory response syndrome (SIRS) with multiple organ dysfunction, shock, renal failure, acute respiratory distress syndrome, disseminated intravascular coagulation, massive gastrointestinal bleeding, and systemic or intra-abdominal infection [44]. In adults, the percentage of necrosis seen on CT scan and failure of pancreatic tissue to enhance on CT scan (suggesting necrosis) predicts severity of the disease [14, 41].

There are now two defined, distinct phases of severe AP, which overlap: the early phase, which usually lasts only 1 week or so, and the late phase, which can persist for weeks to months. The early phase is characterized by the SIRS and/or the compensatory anti-inflammatory syndrome (CARS), which can predispose to infection. Adult patients with severe AP that develops within the early phase (first week) have 36–50% mortality [14]. Development of infected necrosis later in the course of the disease in patients with severe AP also has an extremely high mortality [45, 46]. The late phase is characterized by the persistence of systemic signs of ongoing inflammation, the presence of local and systemic complications, and/or transient or persistent organ failure. Local complications include peripancreatic fluid collections, pancreatic and peripancreatic necrosis, pseudocysts, and walled-off necrosis. By definition, the late phase occurs only in patients with moderately severe or severe AP [41, 47].

Treatment

While the mainstay of treatment for AP is mainly supportive, efforts have been made in recent years to provide more specific recommendations about fluid provision, monitoring, pain management, and nutrition.

Intravenous fluid therapy remains a mainstay in the treatment of AP. In severe pancreatitis, fluid resuscitation in the first 24 hours is critical [48]. Progression to severe AP is thought to be secondary to alteration in the microcirculation of the pancreas by events including hypovolemia, increased capillary permeability, and formation of microthrombi. While consensus on type of fluid remains controversial, crystalloids are preferred over colloids for initial resuscitation. The most recent pediatric consensus statement notes theoretical and potential clinical advantages favor lactated Ringer's solution over normal saline based on adult data, but pediatric data are still lacking [13]. The rate of fluid administration continues to be controversial. Given the goal of improved pancreatic microcirculation, rates of 1.5 to 2 times maintenance over the first 24–48 hours have been recommended. Intravascular fluid losses due to third spacing and losses through an NG tube can rapidly lead to dehydration and cardiovascular instability. So not only is volume expansion important but maintenance of intravascular volume is critical. Vital signs and markers such as BUN, creatinine, and urine output should be closely monitored in the acute period to evaluate for volume overload and acute kidney injury.

The aims of medical management are to relieve pain and restore metabolic homeostasis. Analgesia should be given in adequate doses. A 2013 Cochrane review stated that opioids may be an appropriate choice for pain in AP in adults and may decrease the need for supplementary analgesia [49]. They found that opioids did not increase the risk of pancreatitis complications or significant adverse events. Current consensus guidelines recommend intravenous opioids be used for pain not responding to either acetaminophen or NSAIDs.

In *mild acute pancreatitis,* nasogastric suction is needed infrequently but is useful in patients who have significant vomiting. While vomiting, the patient should be maintained with nothing by mouth. Refeeding can commence when vomiting has resolved. Early refeeding decreases the complication rate and length of stay [13, 40]. While traditional, there are no data to support the use of a low-fat diet. It is not necessary to follow laboratory tests such as serum lipase daily. Recovery is usually complete within 4–7 days.

Analgesia is given as needed. Antibiotics are used to treat infected necrosis, but prophylactic antibiotics are not recommended. Infected necrosis does not occur until the second week of pancreatitis [46].

Endoscopic therapy can be of benefit when pancreatitis is caused by anatomic abnormalities, such as strictures or stones [50]. The endoscopist can dilate the sphincter of Oddi and extract impacted biliary tract stones. This is especially important when the patient has developed cholangitis.

Historically, patients diagnosed with AP were designated as NPO and placed on parenteral nutrition. A recent pediatric guideline recommends enteral nutrition initiation as early as possible [13]. Prompt initiation of EN is thought to prevent bacterial translocation, decrease cytokine response, as well as decrease incidence of gastroparesis and intestinal ileus.

Early EN is begun, whether by mouth, nasogastric tube, or nasojejunal tube, in patients intolerant to oral or nasogastric feedings. When initiated within 2–3 days of onset of pancreatitis, enteral feeding reduces the length of hospitalization, complication rate, and increases survival in adult patients with severe AP [13]. Supplemental parenteral nutrition may be needed in some patients.

Measurement of gastric residuals and abdominal circumference are of limited value and should be discouraged. Residual volume relates to infusion rate. High residuals are common early in enteral nutrition. Gastric distension may be from gas-filled loops of bowel. A rise in serum amylase and lipase is typically associated with enteral feeding. These rises do not imply worsening of the pancreatitis and are not reasons to stop enteral nutrition.

When a patient has persistent fever into the second week of illness or develops fever later, a CT scan is required [14]. When necrotic tissue is seen, fine needle aspiration and culture is indicated [14]. The use of antibiotics in sterile necrosis is not recommended. Antibiotics of choice for infected pancreatic necrosis and abscess are those that are known to penetrate the pancreas including carbapenem, quinolones, and metronidazole [14].

In *traumatic pancreatitis*, surgery is frequently required. In children, surgical therapy of nontraumatic AP may include drainage and resection of necrotic material or abscesses. In stable patients, surgery is deferred at least 2–4 weeks to allow differentiation between necrotic and viable tissues [14]. In gallstone pancreatitis, it is recommended that cholecystectomy be performed before hospital discharge to prevent recurrent episodes [14].

Complications

Most children with acute uncomplicated pancreatitis will do well and recover within 4–7 days. However, about 20% of children with AP will develop a complication, which may prolong the hospital stay or require intervention [13]. Complications are divided into local and systemic. Local complications include acute peripancreatic fluid collections, pancreatic pseudocysts, acute necrotic collections, walled-

off necrosis, splenic/portal vein thrombosis, colonic necrosis, retroperitoneal hemorrhage, and gastric outlet dysfunction. Local complications should be suspected when there is persistent abdominal pain, fever/chills, or organ failure and should prompt cross-sectional imaging to search for these complications [14, 34, 41]. Acute peripancreatic fluid collections are not associated with necrotizing pancreatitis, remain sterile, and usually resolve without intervention. A fluid collection persisting more than 4 weeks is likely developing into a pancreatic pseudocyst. A pancreatic pseudocyst is a peripancreatic or intrapancreatic fluid collection or collections containing no solid material surrounded by a well-defined wall. Acute peripancreatic fluid collections and acute necrotic collections occur within the first 4 weeks of AP, while pancreatic pseudocysts and walled-off necrosis usually arise more than 4 weeks after the onset of AP [13, 39].

Systemic complications occur from the systemic inflammatory response to AP and may be further exacerbated by the need for fluid resuscitation. Systemic complications include de novo occurrence of renal, circulatory, or respiratory organ failure or exacerbation of serious preexisting comorbidities [39, 51].

It should be noted that 15–20% of patients will go on to develop acute recurrent pancreatitis [52].

Future

Over the past 20 years, as the incidence of AP has risen, the percentage of children with idiopathic AP has decreased, with the recognition of more genetic causes, the wide availability of ERCP, and with the advent of new technologies such as MRCP and endoscopic ultrasound. Greater understanding of the pathophysiology of pancreatitis has led to improvement in therapy such as early introduction of enteral feeding and rapid fluid resuscitation in severe pancreatitis.

Since there are limited controlled studies on any of the various aspects of the treatment of AP in children, nearly all the current recommendations are based on publications describing adults. Particular areas that are in need of investigation include a validated scoring system to predict severity of AP in children, fluid resuscitation, use of antibiotics, antioxidants, probiotics, timing of nutrition therapy, and the risks and benefits of advanced modalities such as ERCP and endoscopic ultrasound.

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Chronic and Hereditary Pancreatitis

35

Elissa M. Downs and Sarah Jane Schwarzenberg

Introduction

Chronic pancreatitis is the continuation of progressive inflammatory damage to the pancreas that has resulted in irreversible acinar and ductal change, which can eventually cause corresponding loss of endocrine or exocrine function. Children with acute pancreatitis may progress from their initial attack to chronic pancreatitis in a median timeframe of 3.79 years [1] or as little as median 0.7 years [2]. Certain genetic risk factors, such as PRSS1 and SPINK1, may be more likely to present with findings of chronic pancreatitis [3] and children with PRSS1 mutations may progress to chronic pancreatitis more rapidly than those without [1].

Progressive irreversible pancreatic damage leads to intractable pain [4] and significantly impaired quality of life [2, 5]. The burden of disease is high across all age groups, with no significant differences in emergency department evaluations, hospital stays, or missed days of school/work [4] between children and adults. Children with chronic pancreatitis report frequent opioid usage, with high health care utilization, and impaired quality of life [6].

While over time the incidence of both acute and chronic pediatric pancreatitis appears to be rising [7, 8], this is likely due to greater awareness of the burden of pancreatic disease in children. Most recently, cohort studies in the United States have demonstrated a plateau to a decrease in incidence of chronic pancreatitis [9] in pediatrics to 1.9 per 100,000 persons per year and in adults to 24.7 per 100,000 persons per year. Global incidence is less well-documented overall [10], and this is especially true for pediatric chronic pancreatitis.

In pediatrics, our understanding of acute recurrent and chronic pancreatitis has been advanced by several country-wide and global consortia that have combined to produce large enough cohorts to achieve high-quality data collection. Examples include INSPPIRE (*IN*ternational *S*tudy group of *P*ediatric *P*ancreatitis: *I*n search for a cu*RE*), an international multicenter study of acute recurrent and chronic pancreatitis in children [11], and the APPLE study of the Hungarian Pancreatic Study Group, a multicenter study of the course of pediatric pancreatitis with emphasis on the genetic risk factors [12].

Pathophysiology

Models of pancreatic injury suggest a sentinel acute pancreatitis event (SAPE) that, in some children, initiates a pathway toward chronic inflammation. With this event, pancreatic stellate cells can then drive fibrosis from recurrent acinar cell injury [13]. This model has allowed identification of different events or risk factors that lead progression from acute, to acute recurrent, to chronic pancreatitis.

Risk Factors

The most commonly used classification system for risk factors for chronic pancreatitis is the TIGAR-O risk factor classification system [14], which includes *T*oxic/metabolic, *I*diopathic, *G*enetic, *A*utoimmune, *R*ecurrent/severe acute pancreatitis, and *O*bstructive etiologic risk factors (see Table 35.1). Over 20% of children with chronic pancreatitis have more than one risk factor for its development [15]. Depending on the extent of testing done, the exact etiology may, in many cases, only be partially known [14].

As children progress through the stages of acute, to acute recurrent, and then to chronic pancreatitis, proportionality of underlying risk factors shifts toward genetic disease: 73% of children with chronic pancreatitis in recent INSPPIRE cohort assessments were identified as having at least one gene mutation compared to 48% of those with acute recurrent

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pancreatitis [3]. In all likelihood, this proportion is falsely low as not all children had a full complement of testing performed [3] and our understanding of pathogenic genetic variants continues to expand over time.

Obstructive risk factors share similar frequency between acute recurrent and chronic pancreatitis and can be found in up to 33% of children [3, 4]. These risk factors may include pancreas divisum, gallstones, biliary cysts, or an anomalous pancreaticobiliary junction among others. The percentage of those with pancreas divisum increases in chronic pancreatitis as compared to acute recurrent pancreatitis, but was not statistically significant in one cohort [3]. The presence of pancreas divisum should not preclude evaluation for other risk factors [16].

Toxic and metabolic risk factors may include exposure to medications such as L-asparaginase [17], or hypertriglyceridemia [18, 19]. While exposure to second-hand smoke may occur in up to 10% of those with chronic pancreatitis [3], different pediatric cohorts demonstrate active smoking is much less common (2–7%) as is active alcohol intake (1–4%) [3, 4] especially when compared to the adult chronic pancreatitis population.

Table 35.1 TIGAR-O risk factor classification system

Toxic/metabolic
Alcohol intake
Tobacco smoking
Hypercalcemia
Hyperlipidemia
Chronic renal failure
Medications (L-asparaginase, valproic acid, azathioprine/mercaptopurine, steroids)
Idiopathic
Genetic
PRSS1
CFTR
SPINK1
Others
Autoimmune
Recurrent and severe acute pancreatitis
Obstructive
Pancreas divisum
Gallstones
Biliary cysts
Anomalous pancreaticobiliary junction

After Etemad and Whitcomb [14]

Diagnosis

Establishing the Diagnosis of Chronic Pancreatitis

Standards for the clinical diagnosis of acute, acute recurrent, or chronic pancreatitis were suggested by the INSPPIRE study group in an effort to standardize definitions to guide further research and recommendations into disease management [20]. For a diagnosis of chronic pancreatitis to be established, one of the three conditions must exist: (1) abdominal pain consistent with pancreatic origin and imaging findings suggestive of chronic pancreatic damage; (2) evidence of exocrine insufficiency and suggestive imaging findings; or (3) evidence of endocrine insufficiency and suggestive imaging findings.

Imaging findings may include changes of the duct (irregularity, intraductal filling defects or calculi, stricture, dilatation) or the gland itself (enlargement, atrophy, irregular contour, calcifications) [20, 21] (see Figs. 35.1 and 35.2).

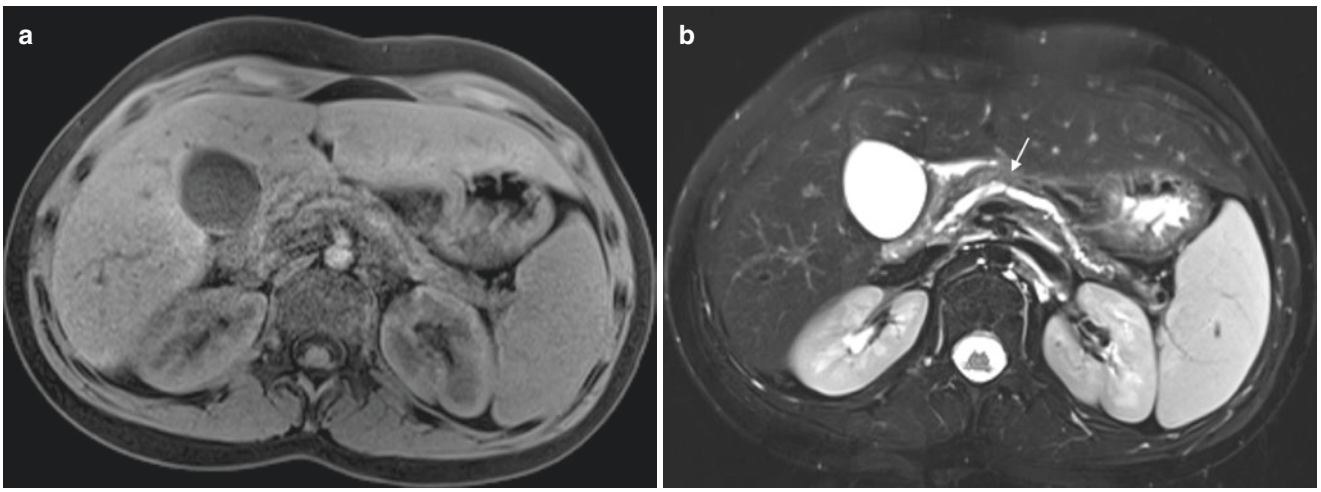

Fig. 35.1 Imaging changes in chronic pancreatitis: Axial (**a**) T1- and (**b**) T2-weighted images with fat saturation. The gland is overall atrophic with isointense to mildly hypointense T1 signal relative to the liver. Notice the abnormally distended pancreatic duct with focal stricture (arrow)

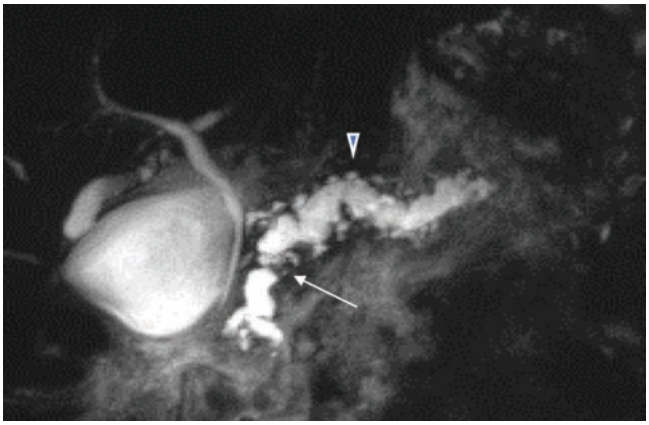

Fig. 35.2 Imaging changes in chronic pancreatitis: On this MIP (maximum intensity projection) image of the pancreatic duct, notice the diffuse ductal enlargement, side branch distention (arrowhead), and focal stricture at the head-proximal body junction (arrow)

Exocrine insufficiency, as diagnosed by fecal elastase-1 <100ug/g stool or a coefficient of fat absorption <90% on 72 hour fecal fat collection [20], will be further discussed below. The diagnosis of endocrine insufficiency follows 2006 World Health Organization criteria, with fasting glucose ≥126 mg/dL (7 mmol/L) or ≥200 mg/dL (11.1 mmol/L) after glucose load [20, 22].

The evaluation of a child for chronic pancreatitis may be difficult. Little evidence guides this process, although expert opinion is available [16]. Choice of imaging depends on many factors, including the quality of the imaging technique, the degree of ionizing radiation, and the possible need for sedation or intubation.

Abdominal ultrasonography (US) is the imaging modality of choice as it is noninvasive, readily available, does not require sedation, and lacks ionizing radiation. US may be more suitable for children given less abdominal fat versus adults as well as better acoustic windowing from the left hepatic lobe [23]. US findings in chronic pancreatitis may include dilated pancreatic duct, calcifications, pseudocysts, and gland atrophy.

Advanced cross-sectional imaging should be considered to better delineate changes seen with chronic pancreatitis, such as dilation and strictures of the pancreatic duct, side branching, and calcific change.

Magnetic resonance cholangiopancreatography (MRCP) is preferred over computed tomography (CT) scanning and its ionizing radiation exposure. However, MRCP may have limitations including inability to visualize small caliber ducts in young children, and sedation might be required to minimize respiratory artifact given longer duration of scanning time [24]. In a large study of pediatric patients with chronic pancreatitis, the sensitivity and specificity of MRCP was 77.1% and 50%, respectively, with diagnostic accuracy of 73% [25], which improved with older teens and more severe disease.

Use of secretin stimulation may improve the detection of more subtle changes of early chronic pancreatitis and improves the sensitivity of MRCP [26]. In a study of children with idiopathic chronic pancreatitis, secretin administration increased the ability to detect dilated side branches, and an irregular or narrow duct as well as pancreas divisum [21]. While changes after secretin stimulation may be statistically significant, it remains a quantitatively small change, so the overall usefulness may be limited [27].

Cross-sectional imaging may also correlate with histopathology. In a study of adults with noncalcific chronic pancreatitis, the number of features on secretin-stimulated imaging significantly correlated with more severe fibrotic histopathology [28].

Endoscopic retrograde cholangiopancreatography (ERCP) for diagnosis of chronic pancreatitis has decreased over time given its invasive nature, need for anesthesia, exposure to radiation, complications (including post-ERCP pancreatitis) [26, 29], and potential limited availability in pediatrics [25]. However, it may better identify, and allow intervention for, pancreas divisum [30].

Endoscopic ultrasound (EUS) performed by appropriately trained endoscopists can be safely and effectively performed in children over 15 kg using standard adult equipment, but still requires general anesthesia [31]. EUS can demonstrate even subtle ductal and parenchymal changes of early chronic pancreatitis [32] and also allows fine-needle aspiration or biopsy if needed. While the Rosemont criteria of EUS changes can be used to diagnose chronic pancreatitis in adults, no such criteria exist in children [31].

Establishing Risk Factors for Chronic Pancreatitis

Once a diagnosis of chronic pancreatitis is made, further testing is necessary to establish the risk factors for the chronic pancreatitis in the child. This may both guide therapy and allow appropriate counseling for the child and other family members.

Following the TIGAR-O system, studies to examine risk factors may include the following:

1. Careful history to uncover possible medications [33, 34] or metabolic disease [33, 35] associated with chronic pancreatitis.
2. Genetic studies to evaluate risk factors for chronic pancreatitis. Note that careful consent for such studies, an explanation of any results, will require a genetic counselor familiar with genetic associations with chronic pancreatitis.

Genetic testing should be performed for all children with otherwise idiopathic pancreatitis and no known risk factors after their second episode of pancreatitis. With a known family history of pancreatitis, genetic testing should be performed after an initial episode.

While target testing can be performed for a known gene, it is possible to have more than one genetic risk factor; in two separate INSPPIRE cohort studies, 9 of 51 children (17.6%) with hereditary pancreatitis had multiple genetic mutations identified [2] in one study and 17 of 112 children (15%) in another [3].

Our knowledge of the pathophysiology of the currently identified genetic mutations comes from their roles in the regulation of trypsin in the pancreas [36].

PRSS1 Mutations in the cationic trypsinogen gene PRSS1, inherited in an autosomal-dominant manner, lead to gain-of-function mutations and overactivation or under/inactivation of trypsin. Early-onset pancreatitis (<6 years old) is associated with PRSS1 [37]. Those with PRSS1 hereditary pancreatitis may be more likely to present with chronic pancreatitis versus acute recurrent pancreatitis [3]

SPINK1 Mutations in the serine protease inhibitor, Kazal type 1 gene SPINK1, may lead to unchecked intrapancreatic trypsin activity and are likely to be clinically important when another process is causing premature activation of trypsin and inflammation [38, 39].

CFTR Mutations in the cystic fibrosis transmembrane receptor gene CFTR may alter bicarbonate secretion [40, 41] and cause duct obstruction due to thickened secretions [42]. Pancreatitis can be an initial presenting sign of cystic fibrosis as these patients may be more likely to have a milder cystic fibrosis phenotype with less severe CFTR dysfunction and may not be identified by newborn screening [43].

Others Additional genetic mutations in trypsinogen regulation have been identified, including chymotrypsin C (CTRC), and the calcium-sensing receptor (CASR). Early-onset pancreatitis has also been associated with CTRC gene mutations [37]. The carboxyl ester lipase (CEL) enzyme expressed in pancreatic acinar cells may lead to protein misfolding that causes a syndrome of endocrine and exocrine dysfunction [44].

3. The diagnosis of autoimmune pancreatitis is challenging in children, as the clinical presentation can be different in children versus adults, and they rarely are positive for IgG4. Diagnosis may be established with combination of clinical symptoms (including acute abdominal pain, obstructive jaundice, and weight loss) and abnormal cross-sectional imaging (such as focal gland enlargement, main pancreatic duct irregularity, distal bile duct stricture) [45].
4. Obstructive risk factors are generally evaluated by MRCP. These risk factors may include pancreas divisum, gallstones, biliary cysts, or an anomalous pancreaticobiliary junction among others as previously discussed

Evaluating the Severity of Chronic Pancreatitis

Complications of chronic pancreatitis include pain and exocrine and endocrine insufficiency. Pain, as a subjective sensation, is difficult to measure. In general, evaluation includes some measure of function (school attendance, participation in sports, and social activities). Pain should be assessed at each clinic visit.

Testing for exocrine and endocrine function should be done yearly [16].

Exocrine Pancreatic Function Assessment

Chronic pancreatitis is the most common cause of exocrine pancreatic insufficiency (EPI) in adults, while cystic fibrosis is the most common cause in children. EPI causes fat malabsorption leading to steatorrhea, weight loss, and nutritional deficiencies. Increased fecal fat may only manifest when the functional reserve of the pancreas falls below 10% [46].

EPI can be measured by several methods. Direct measurement has long been considered the "gold standard" due to high levels of sensitivity and specificity, but is expensive, time consuming, and may only be performed at specialized centers [47].

Direct exocrine pancreatic function testing Older direct methods of pancreatic function testing use an enteric tube to collect pancreatic secretions stimulated from administration of cholecystokinin (provokes enzyme secretion), secretin (provokes bicarbonate production), or both [48].

Variations in these stimulation tests over time have resulted in procedures that are more feasible and better tolerated by patients. These include endoscopic and radiologic studies.

Endoscopic pancreatic function testing (ePFT) has comparable performance to the previous secretin-stimulated Dreiling test. The endoscopic test has greater overall availability, less associated cost, decreased procedure time, and decreased radiation exposure [49]; however, standard protocols and references have not been developed and it does require general anesthesia [50].

Radiologic advancements include a secretin-enhanced MRI that may even allow mild EPI to be detected [51, 52], but would require sedation in younger patients.

Indirect exocrine pancreatic function testing While multiple older methods of indirect pancreatic function testing exist, current commonly used methods to detect EPI include the coefficient of fat absorption (CFA), immunoreactive trypsinogen (IRT), and fecal elastase-1 (FE-1).

Fecal fat measurement entails a 72-hour stool collection while consuming a fixed fat diet [53]. The CFA can be calculated by comparing exact intake versus excretion. Normal CFA values differ by age, with >85% for infants <6 months, and >93% for older infants, children, and adults [54]. If patients are on supplemental pancreatic enzymes, these need to be discontinued prior to the study. Increased fecal fat excretion can be seen in other conditions such as Celiac disease / villous atrophy [53], Crohn disease, or small intestinal bacterial overgrowth [47].

Serum levels of IRT decline as direct function testing demonstrates a parallel decline in bicarbonate output [55]. However, lower levels can also be seen in patients with chronic pancreatitis with normal exocrine function, making this test less reliable. In studies of IRT on patients with cystic fibrosis and EPI, lower IRT levels were associated with steatorrhea [56] and could be used for monitoring for the development of EPI in those over 7 years old [57, 58].

The level of elastase in the stool (FE-1) is the predominantly used test, even though it has limitations. Testing is via a monoclonal antibody ELISA assay, with normal value defined as >200 micrograms of elastase per gram of stool (ug/g) [59]. The assay is specific to the human enzyme and exogenous pancreatic enzyme supplementation will not interfere [60]. The sensitivity of the test is approximately 100% in severe EPI (FE-1 <100 ug/g stool), 77–100% in moderate EPI, and 0–63% in mild EPI (FE-1 <200 ug/g stool) [61, 62]. FE-1 also does not distinguish between primary and secondary causes of EPI and is unreliable on loose stool [63]. For most purposes, the convenience of FE-1 will outweigh its limitations.

Endocrine Pancreatic Function Assessment

Endocrine insufficiency in chronic pancreatitis can happen independently of exocrine insufficiency. This type of diabetes due to islet cell damage and loss, called pancreatogenic or type 3c diabetes mellitus, may reach higher cumulative levels in those with hereditary pancreatitis versus other forms of chronic pancreatitis [64]. In children with acute recurrent or chronic pancreatitis, pancreatic atrophy, hypertriglyceridemia, or coexisting autoimmune disease were more commonly seen in those with already established diabetes [65], and the rate of diabetes in this cohort was 6%. Adult cohort studies have demonstrated that beta-cell function may exhibit more rapid decline in those that were smokers, had undergone previous surgical drainage, or had calcific pancreatitis [66]. Some of these factors are relevant to the pediatric population, highlighting the need for close monitoring.

Indirect endocrine testing Initial testing of endocrine function can be assessed with fasting blood glucose values [67], although there can be variations in fasting values from day to day. By American Diabetes Association [68] and World Health Organization [22] criteria, a fasting blood glucose in the 100–125 mg/dL range indicates impaired glycemia or prediabetes, and a fasting blood ≥126 mg/dL indicates diabetes.

Glycated hemoglobin, or hemoglobin A1c (HbA1c), gives a measure of average blood glucose over the last 2–3 months. By ADA criteria [68], HbA1c of 5.7–6.4% indicates impaired glycemia or prediabetes, and a value ≥6.5% indicates diabetes.

Direct endocrine testing If impairment in fasting blood glucose or A1c is observed, direct challenge is performed with an oral glucose load of 75 grams. At 2 hours post ingestion, blood glucose values ≥200 mg/dL establish a diagnosis of diabetes [22, 68]. Mixed nutrient ingestion (mixed meal tolerance test, MMTT) can distinguish between type 2 and type 3c diabetes mellitus, with limited pancreatic polypeptide (or c-peptide) elevation from baseline in type 3c or pancreatogenic diabetes [67].

Evaluation of Exacerbations

Once the diagnosis of chronic pancreatitis is established, families must be made aware of the need for evaluation during exacerbations. Some testing may be needed when a child with known chronic pancreatitis comes to clinical attention because of abdominal pain, vomiting, and other signs of an acute exacerbation of pancreatitis. These tests differentiate a flare of pancreatitis from other causes of abdominal pain that may present in similar fashion, such as urolithiasis or urinary tract infection, hepatobiliary disease, constipation, bowel obstruction, celiac disease, peptic ulcer disease, or gastritis/gastroenteritis.

Exacerbation Workup

While the pancreatic enzymes, amylase and lipase, can be assessed during a suspected pancreatitis episode, these levels may be only minimally abnormal or may not be elevated at all in a flare of chronic pancreatitis because of pancreatic atrophy and fibrosis [69].

Additional testing may include a hepatic panel (liver enzymes, bilirubin, alkaline phosphatase, gamma-glutamyl

transferase) to assess for hepatobiliary disease, urinalysis with possible culture to identify kidney stones or a urinary tract infection, and screening for Celiac disease if suspected. Imaging may include a complete abdominal ultrasound to identify hepatobiliary, renal, or genitourinary disease or an abdominal x-ray for stool burden or an obstructive bowel gas pattern.

Additional blood work should be pursued to determine potential etiologic risk factors (such as liver enzyme testing, triglycerides, calcium) or for predictors of severity (such as albumin, white blood cell count, blood glucose, lactate dehydrogenase, hematocrit, blood urea nitrogen). Many predictive models of severity have been trialed in both adults and children, but none have enough positive predictive power to use as a single-step criterion of risk for severe pancreatitis and are suboptimal in pediatrics [70].

Treatment

Medical

Pain Management

The chronic abdominal pain that develops in the setting of chronic pancreatitis is multifactorial and requires management from both a pharmacologic and nonpharmacologic standpoint. An inclusive regimen should address components of nociceptive pain, neuropathic pain, and central sensitization of pain [71], but should also include an interdisciplinary approach with psychologists/psychiatrists, integrative medicine providers, and physical/occupational therapists [72] (see Table 35.2).

Current pharmacologic interventions include nonopioid analgesics and opioids to address nociceptive pain. First-line therapy should include nonsteroidal anti-inflammatory agents and acetaminophen/paracetamol. For severe or refractory pain, step-up therapy with opioids can be employed.

Frequent opioid use may be more likely in those children that were older at age of initial pancreatitis diagnosis, were more likely to have the PRSS1 gene mutation, had exocrine pancreatic insufficiency, had constant or severe pain, and had pain that impacted all functional domains [6].

For opioid-sparing effects, the regimen should include neuropathic pain–modifying agents such as antidepressants, and gabapentinoids. Adults with chronic pancreatitis treated with pregabalin had significant pain relief and reduced need for opioid medication [73], although not extensively studied in pediatrics.

Other pain management modalities have been employed to address visceral pain, such as celiac nerve blocks. These can be performed via CT or EUS; injection agents can include alcohol or steroid. However, studies in adults are mixed over a limited follow-up timeframe [74], and only case reports exist in pediatric chronic pancreatitis; they are generally not recommended for children [75].

Table 35.2 Multidisciplinary chronic pancreatitis team

Provider[a]	Role
Gastroenterologists	Pancreatitis and complication management, exocrine insufficiency treatment
Endocrinologist/diabetes educators	Diagnosis and management of endocrine dysfunction
Pain and palliative care providers	Complex pain management
Psychologists/psychiatrists	Adjustment to chronic disease or comorbid mood disorders
Physical therapists	Therapy for physical deconditioning related to chronic disease
Integrative medicine specialist	Nonpharmacologic pain management
Social Work	Connection to resources for patient and family
Child/family life specialist	Assist with procedural-related anxiety
Dietitian	Monitor growth, provide nutrition counseling
Nurse coordinator	Help families to navigate complex care
Surgeon	Surgical management of chronic pancreatitis

[a]Roles are overlapping and a coordinated team approach to management is ideal

Pancreatic Enzyme Replacement Therapy

Supplemental pancreatic enzymes are critical in patients with chronic pancreatitis that has progressed to exocrine insufficiency as a way to optimize fat and vitamin absorption and decrease pain related to malabsorption. A recent meta-analysis of randomized clinical trials limited to those with EPI found that supplemental enzymes decreased fecal fat excretion and improved abdominal pain and thus were clearly indicated [76]. A previous meta-analysis found equivocal results for supplemental enzymes on pain, quality of life, and fecal fat excretion but included those with and without evidence of EPI [77]. It does not appear to be beneficial to provide supplemental enzymes for chronic pancreatitis pain relief in those without EPI [78].

Others

Antioxidant therapy has been hypothesized as a way to control oxidative stress to the pancreas and minimize injury from free radicals; however, an older meta-analysis failed to show benefit [78]. More recent placebo-controlled clinical trials have had mixed results. In a cohort of older adults with chronic pancreatitis (largely alcoholic in origin, with high percentage of smokers), antioxidant therapy did not demonstrate significant improvement in pain scores, quality of life, decreased opiate use, or hospital visits [79]. While in a cohort of younger adults, painful days and analgesic use was

decreased [80], leading the authors to conclude there was benefit.

Psychology

Pediatric psychologists trained in cognitive behavioral therapy (CBT) can teach coping skills, address concomitant mood disorders, and improve overall functioning [72]. An ongoing clinical trial of an internet-based CBT approach will determine the efficacy of this approach on pain management and quality of life specific to children with acute recurrent or chronic pancreatitis [81], especially given access issues to trained health psychologists [72].

Endoscopic

As discussed above, ERCP has largely switched from a diagnostic to a therapeutic modality for obstructive pancreaticobiliary disease and chronic pancreatitis [82]. ERCP with sphincterotomy, ductal dilatation, stenting, and/or stone removal in accomplished hands can lead to improvement in pancreatic drainage and pain as well as decreased episodes of acute pancreatitis [83, 84]. It is a common therapeutic intervention in children with chronic pancreatitis, with 65.8% in the INSPPIRE cohort undergoing at least one ERCP [85]. ERCP appears to offer the most benefit to those children with pancreas divisum or obstructive stones [30].

Notable complications include post-ERCP pancreatitis [26, 29], bleeding, perforation, and infection. A limited trial of endoscopic intervention can be offered in appropriate patients, but step-up surgical therapy should be pursued if patients are not responding [86].

Surgical

Surgical decision-making and technique should be tailored to the patient's disease presentation: pancreatic anatomy, duct size/dilation, or genetic and other risk factors [86].

Cholecystectomy

Given the rise of obesity in the pediatric population and associated biliary disease, adolescents with gallstone pancreatitis should undergo cholecystectomy to reduce the recurrence risk and progression to chronic pancreatitis [87]. When cholecystectomy is delayed, ongoing abdominal pain and further episodes of pancreatitis have been observed [87, 88]. In adults with otherwise idiopathic etiology of acute pancreatitis, cholecystectomy for possible microlithiasis led to a statistically significant decrease in pancreatitis recurrence [89]; however, it is unclear if this strategy would provide benefit in those with already established chronic pancreatitis.

Drainage and Partial Resection Procedures

Procedures such as a lateral pancreaticojejunostomy (Puestow) are indicated for large duct disease for decompressive drainage, but children may be more likely to present with small duct or diffuse disease [90]. In a single center case series of children with chronic pancreatitis undergoing lateral pancreaticojejunostomy, 58.3% of patients reported improvement in abdominal pain over median follow-up of almost 3 years [91], leaving a large percentage still with no improvement in, or recurrence of, abdominal pain.

A pancreaticoduodenectomy (Whipple) resection is indicated for a predominant pancreatic head mass, which is less commonly seen in children but may be performed for associated obstructive jaundice [92] or pancreatic malignancies [93]. Procedures that combine drainage and resection (such as Beger or Frey) may offer more durable results [94, 95], but this has not been seen in all pediatric case series [86].

These procedures should be avoided in children with hereditary pancreatitis [90] given more diffuse pancreatic involvement. In addition, because any surgical resection of the pancreas will lead to lower islet cell yield and increased risk of insulin dependence in the future [96, 97], these procedures should be avoided in those felt to be a candidate for total pancreatectomy islet-auto transplant surgery (TPIAT) [86].

Total Pancreatectomy, Islet Autotransplantation

When patients have failed to respond to maximal medical, endoscopic procedures, or other surgical procedures, total pancreatic (TP) resection can be considered to remove the nociceptive source of chronic unrelenting pain and improve quality of life. This will lead to brittle diabetes if completed alone; thus, it is coupled with islet autotransplantation (IAT) to minimize development of pancreatogenic diabetes. Details of the surgical technique are described in depth in the first large series of TPIAT in children [96], but usually includes total pancreatectomy, partial duodenectomy, Roux-en-Y duodenojejunostomy, cholecystectomy, choledochojejunostomy, splenectomy, the islet infusion, and temporary gastrojejunostomy tube placement. Refinements to the surgical process have occurred over time [98], especially in regards to islet isolation [99].

This is a complex process involving a multidisciplinary team to ensure that patients are appropriate candidates for surgery and that care is optimized prior to surgery. At the University of Minnesota, TPIAT candidates must meet the following criteria: irreversible cause of acute recurrent or chronic pancreatitis leading to chronic pain of greater than 6 months duration, failure of maximal therapies, significantly

impaired quality of life and/or opioid dependence, and adequate islet cell function [96, 97].

Pain relief Pain relief post-TPIAT is accomplished in a high percentage of individuals. In a cohort of those with hereditary pancreatitis, 90% were pain-free post-TPIAT, and improvement in pain was sustained through 10 years of follow-up [100].

Persistent abdominal pain after surgery may be due to gastrointestinal dysmotility and/or central sensitization of pain [101]. Persistent opioid use post-TPIAT has been associated with a prior Whipple or pancreas divisum, greater than 3 previous stents placed during ERCP, and more than 5 years on opioids prior to surgery [98].

Insulin independence Insulin independence has been achieved post-TPIAT. Factors associated with increased insulin independence include female gender [102], lower body weight [102], and, most importantly, more islet equivalents transfused per kilogram of body weight [98, 102, 103]. Prior surgical drainage or partial resection diminished islet yield and was associated with a lower likelihood of insulin independence [96, 97]. Other factors associated with lower islet yield include years with pancreatitis, the surgical era of TPIAT, and, most importantly, pancreatic fibrosis [100].

In the largest series of long-term outcomes in both children and adults after TPIAT, insulin independence (full graft function) or minimal insulin use (partial graft function) was documented at 77% at 1 year, 60% at 5 years, and 47% at 10 years post-TPIAT, which includes 20% that were fully insulin independent at 10 years post-TPIAT [104]. From this study, children (<18 years old) had statistically significant greater likelihood of achieving insulin independence, with odds ratio >9. In a study of TPIAT in children, 82% of young children (under 8 years old) were insulin independent compared to 41% in those over 8 years old [105].

Quality of life Quality of life impairments are seen in both mental and physical domains in children with chronic pancreatitis, far below the normal population [97], and were associated with higher levels of fatigue [5]. After TPIAT, health-related quality of life significantly improved in dimensions of physical and social functioning as well as bodily pain. Physical and emotional summary component scores completely normalized after TPIAT; this improvement was consistently observed through 2 years follow-up post-TPIAT. Functional improvements are noted in better school attendance and decreased activity limitations [96]. In the largest series of long-term outcomes after TPIAT, health-related quality of life continued to improve even at 10 years out from surgery [104].

Younger children (under 8 years old) with lower amounts of pancreatic fibrosis may be good candidates for TPIAT. They have excellent outcomes with high rates of pain relief (100% off opioids at latest study follow-up) and insulin independence (82% vs. 41% in those over 8 years old) [105]. Thus, it is important to consider referral for TPIAT early in young patients with established genetic/hereditary pancreatitis with refractory pain [100, 105].

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Congenital Disorders of Intestinal Electrolyte Transport

36

Lavinia Di Meglio and Roberto Berni Canani

Introduction

Congenital diarrheal disorders (CDDs, [OMIM] 251,850) are a group of rare chronic enteropathies characterized by a heterogeneous etiology with a typical onset early in life. In the first few weeks of life, patients affected by CDD usually present with severe diarrhea that within a few hours leads to a life-threatening condition secondary to massive dehydration and metabolic acidosis (or alkalosis in the case of congenital chloride diarrhea, CLD) [1]. The number of conditions included within the CDD group has gradually increased, and many new genes have been identified as functionally related to CDD, opening new diagnostic and therapeutic perspectives [1].

We have proposed a CDDs classification in four groups concerning the main defect:

(i) Defects of digestion and absorption of nutrients and electrolytes
(ii) Defects of enterocyte structure
(iii) Defects of enteroendocrine cells differentiation
(iv) Defects of intestinal immune-related homeostasis

In the context of the first group, the congenital disorders of intestinal electrolytes transport are a subset of diseases characterized by early clinical presentation due to autosomal-recessive defect. These are challenging clinical conditions for pediatric gastroenterologists because of the severity of the clinical picture and the broad range of conditions in the differential diagnosis. Frequently, abnormal fluid absorption begins in utero, manifesting itself as maternal polyhydramnios and/or bowel dilatation. Then soon after birth, patients usually present with severe diarrhea that within few hours leads to a life-threatening condition secondary to massive dehydration and metabolic acidosis/alkalosis [1]. Consequently, these patients require prompt diagnosis and assistance. Milder forms with subtle clinical signs may remain undiagnosed until adulthood when patients developed irreversible complications. In particular, patients with cystic fibrosis (CF) may have a subtle clinical presentation, with prevalent nonintestinal symptoms.

Different mechanisms are responsible for transepithelial ion transport at the intestinal level (Fig. 36.1). In the jejunum, $NaHCO_3^-$ is absorbed via Na^+/H^+ exchange (the secreted H^+ neutralizes an equivalent amount of luminal $NaHCO_3^-$); Cl^- is absorbed passively down this concentration gradient [2]. In the ileum (and, as shown later, also in the proximal colon), NaCl is absorbed via equal rates of Na^+/H^+ and $Cl^-/NaHCO_3^-$ exchanges [2]. At least three Na^+/H^+ exchangers (NHE) have been localized to intestinal brush border membranes and cloned (NHE2, NHE3, and NHE8); NHE2 and NHE3 are found in both small intestine and colon. NHE3 appears to be quantitatively more involved in congenital diarrheal disorders (CDD). NHE2 knockout mouse suffers gastric dysfunction but no intestinal disability has been apprised, whereas the NHE3 knockout mouse suffers from chronic diarrhea. In addition, NHE3 gene mutation results in congenital sodium diarrhea (CSD). A third, Cl^- -dependent NHE is found in crypt cells of the rat distal colon. The NHE first identified in the intestine, NHE1, is present only in the basolateral membrane of enterocytes and is involved in HCO_3^- secretion [3].

Two anion exchangers have also been localized to small-intestinal and colonic brush border membranes and cloned: downregulated in adenoma (DRA) and putative anion transporter 1 (PAT1) [1]. DRA was first cloned from colonic

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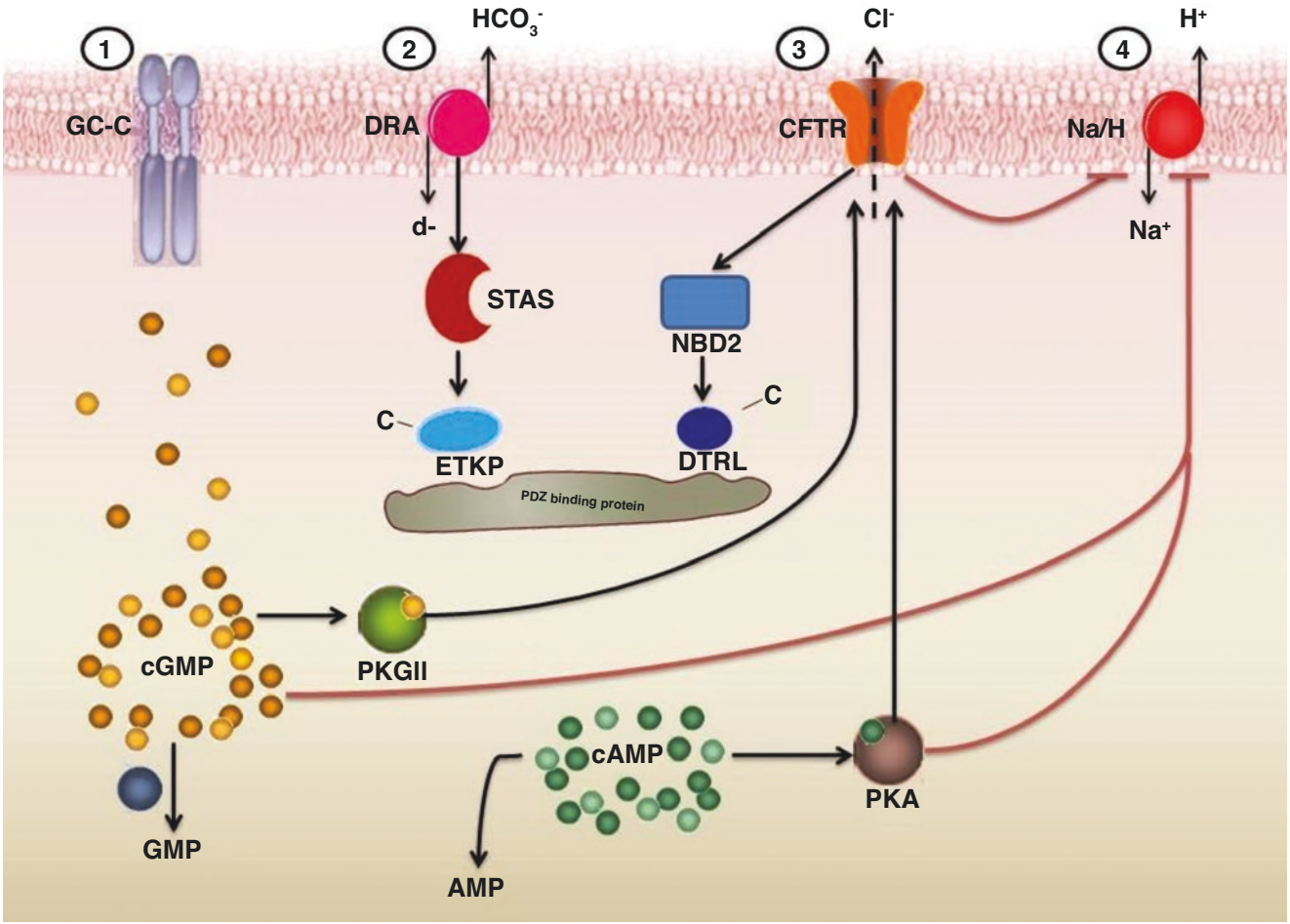


Fig. 36.1 Main pathophysiological mechanisms of congenital disorders of intestinal electrolyte transport
1. Pathophysiological mechanisms of congenital disorders of intestinal electrolytes transport. Familial diarrhea syndrome is due to a mutation in intestinal guanylate cyclase receptor (GC-C) for the endogenous ligands uroguanylin/guanylin. Ligand binding to GC-C increases intracellular levels of cyclic guanosine monophosphate (cGMP). The cGMP activates cGMP-dependent protein kinase II (PKGII). PKGII phosphorylates the cystic fibrosis transmembrane conductance regulator (CFTR), increasing its Cl–-secreting activity and inhibiting electroneutral NaCl absorption. A missense mutation leads to a gain of function, increasing ligand-mediated activation of GC-C and intracellular cGMP levels resulting in chronic secretive diarrhea. 2. Congenital chloride diarrhea is caused by a defect in DRA exchanger, leading to severe watery diarrhea due to Cl – malabsorption. The protein has a C-terminal domain, sulfate transporter, and anti-sigma factor antagonist (STAS) that ensures the correct location of the protein on the apical membrane of enterocytes. The STAS domain also interacts with the R-domain of the CFTR, and it is required for CFTR activation. 3. In cystic fibrosis, there is an alternated activity of CFTR. This membrane protein consists of two membrane-spanning domains, two nucleotide-binding domains (NBDs), and a regulatory domain, which controls channel activity and it functions as a cAMP-dependent chloride channel. 4. Congenital sodium diarrhea probably derives from a defect in apical membrane Na+/H+ exchangers, leading to severe watery diarrhea due to Na + malabsorption
DRA downregulated in adenoma, DTRL Defense Terrain Research Laboratory, cAMP cyclic adenosine monophosphate, AMP adenosine monophosphate, GMP guanosine monophosphate

mucosa; it was found to be downregulated in villous adenomas and carcinomas and, subsequently, was found to incur mutations in the rare "congenital chloride diarrhea." Both DRA and PAT1 are abundant in the duodenum and are present at higher density there than NHE2 and NHE3, suggesting a role in duodenal alkalinization. In the colon, DRA appears to predominate over PAT1. In the descending colon, mineralocorticoid-regulated apical epithelial sodium channels (ENaC) is implied Na^+ absorption [4].

More than two brush border ion exchangers are required, of course, for the enterocyte to engage in transcellular salt absorption. Increased turnover of the Na^+/K^+ pump and the opening of Cl^- and K^+ channels are also necessary, the latter to counteract associated cell swelling, to permit serosal exit of Cl^- taken up from the lumen, and to dissipate the added uptake of K^+ through the pump.

Glucose and Galactose are mainly absorbed by an SGLT1 expressed apically in the brush border of the upper third

small intestinal villi and diffuse out of the cell through the basolateral GLUT2.

SGLT1 uses a Na^+ electrochemical gradient generated by the basolateral Na^+/K^+ ATPase pump: two Na + accompany each glucose molecules [4]. Mutation of SGLT1 is responsible for glucose–galactose malabsorption diarrhea [5]. Fructose is reabsorbed independently by facilitated diffusion process through GLUT5 [4].

The diagnostic approach to CDDs is a multistep process that includes the careful evaluation of the anamnesis and clinical data, results of common laboratory and instrumental procedures, and molecular analysis (Fig. 36.2). The positive familiar history of early-onset chronic diarrhea, polyhydramnios, and/or dilated bowel loops at ultrasound examination during pregnancy are highly suggestive of CDDs. Recently fetal MRI and amniotic electrolytes have shown a potential diagnostic role for congenital sodium (CSD) or chloride losing diarrhea (CLD) [6, 7].

The main symptom is chronic diarrhea (during longlasting than 30 days). In the diagnostic approach, it is important to take into account that infections and food allergy are the most common causes of diarrhea also at this particular

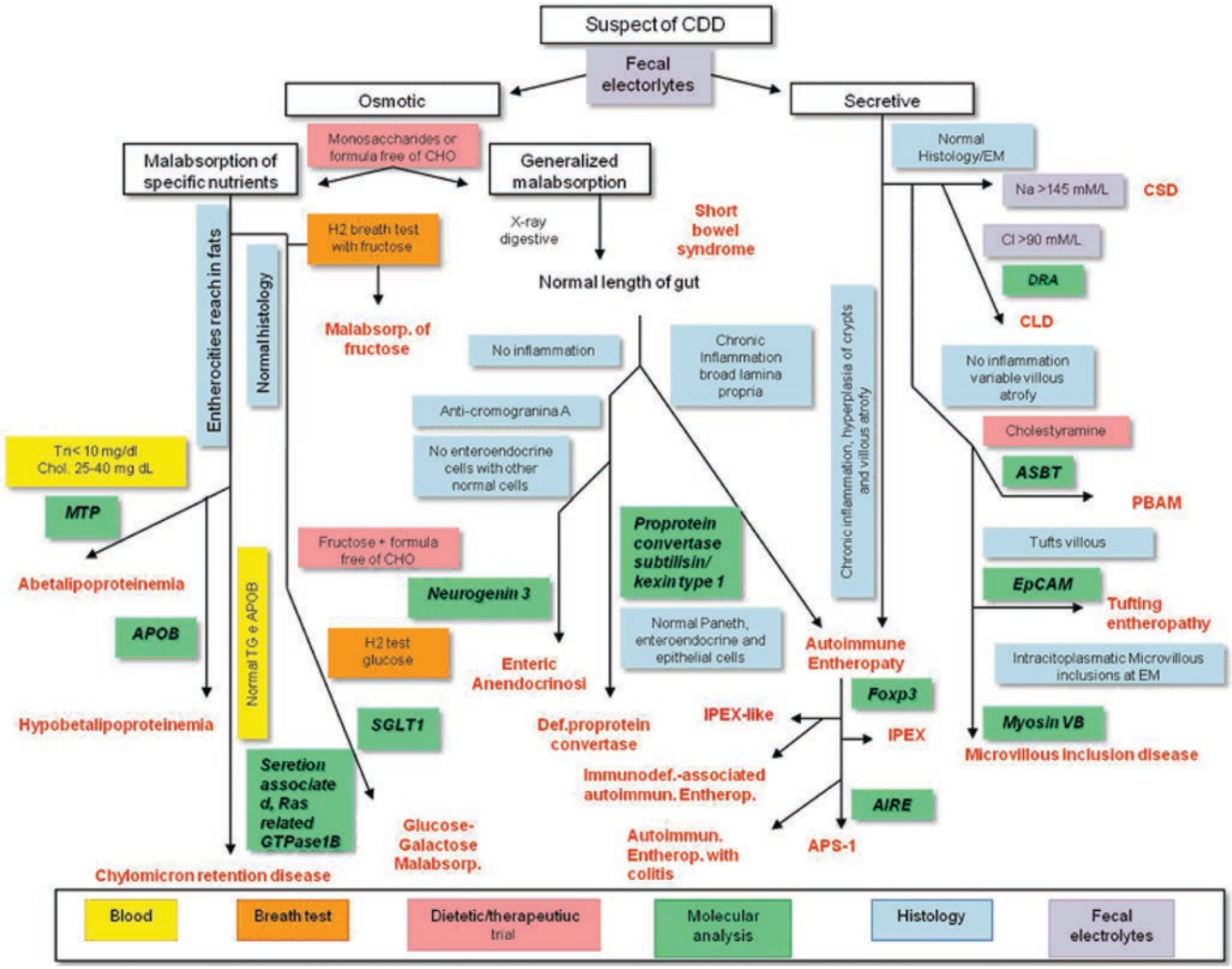


Fig. 36.2 Indications for a modern diagnostic approach to the main forms of CDDs

Indications for an integrated diagnostic approach to the main forms of CDDs. The diagnostic approach is a multistep process that includes the evaluation of anamnesis and clinical data, results of common laboratory and instrumental procedures and molecular analysis. The fundamental step in the diagnostic process of CDDs is the identification of an osmotic or secretory mechanism, leading to diarrhea. Moreover, the determination of stool electrolyte concentration and fecal ion gap is important to discriminate the two mechanisms responsible for CDDs. The next step is laboratory investigation that includes blood gas, blood glucose, ammonium, albumin, triglycerides, and cholesterol, aminoaciduria and the search of reducing substances in the stools, steatocrit, and sweat test. Finally, intestinal biopsy with histologic examination is crucial for the diagnosis of most CDDs. Molecular analysis, when available, is important and could limit invasive procedures

CDD congenital diarrheal disorder, DRA downregulated in adenoma, CLD congenital chloride diarrhea, ASBT apical sodium-dependent bile acid transporter, EpCAM epithelial cell adhesion molecule, PBAM peripheral blood adherent monocyte, IPEX immune dysregulation, polyendocrinopathy, enteropathy, X-linked, MTP media transfer protocol, APOB apolipoprotein B, SGL2 sodium-glucose transport, AIRE autoimmune regulator

age [8], and that these conditions, together with malformations of the gastrointestinal (GI) tract, should be considered as a primary diagnostic hypothesis in an infant with chronic diarrhea [9]. However, in all cases of chronic diarrhea starting in the first few weeks of life, the presence of a congenital disorder of intestinal electrolyte transport should be excluded. A fundamental step in the diagnostic process is the identification of an osmotic or secretory mechanism leading to diarrhea. In osmotic diarrhea, unabsorbed luminal substances are responsible for the accumulation of fluids in the intestinal lumen and diarrhea significantly improves during fasting; whereas, in secretory diarrhea, fluids are actively secreted in the intestinal lumen and diarrhea continues during fasting [9]. Furthermore, the determination of stool electrolyte concentration and fecal ion gap is important to discriminate the two mechanisms responsible for CDDs (Fig. 36.1). If the ion gap is >50 fecal, the diarrhea is an osmotic diarrhea, in contrast, a low osmotic gap (<50) is typically observed in secretory diarrhea. Direct measurement of Na^+ and Cl^- concentration in the stool may suggest a diagnosis of congenital sodium (CSD) or chloride losing diarrhea (CLD), respectively [9]. When an osmotic mechanism is suspected, the next step of laboratory investigation includes blood gas, blood glucose, ammonium, albumin, triglycerides and cholesterol, aminoaciduria, and the search for reducing substances in the stools, steatocrit, and sweat test (Fig. 36.2). Cystic fibrosis (CF) is suggested by the presence of steatorrhea and confirmed by a positive sweat test. On the other hand, signs of inflammation in children with secretory diarrhea and familiar history of chronic diarrhea justified the suspect of a familiar diarrhea syndrome (FDS). The recent availability of molecular analysis for many of these conditions, such as Next-Generation Sequencing diarrhea-related genes panel, has progressively limited the need for invasive procedures (Fig. 36.2).

Congenital Chloride Diarrhea

Congenital chloride diarrhea (CLD; OMIM 214700) is caused by mutations in the solute carrier family 26 member 3 (SLC26A3) gene, and it is responsible for a life-long secretory diarrhea with high fecal Cl- concentration [10]. The gene, located on chromosome 7.q31, encodes for a transmembrane protein that takes part in the Cl^-/ $NaHCO_3^-$ exchange (Fig. 36.2) [11]. The SLC26A3 is located close to the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR), raising a possibility of CFTR modulation in the pathogenesis of CLD [12]. The protein is composed of 764 amino acids that fold to create a transmembrane glycoprotein with hydrophobic segments that cross the membrane 14 times [13]. The C-terminal domain, STAS (sulfate transporter and anti-sigma factor antagonist), ensures the correct location of the protein on the apical membrane of enterocytes, interacts with the R-domain of the CFTR, and it is required for CFTR activation. Studies have shown that deglycosylation of HA-SLC26A3 may contribute to the pathogenesis of diarrhea associated with CDG (congenital disorder of glycosylation), because oligosaccharides protect HA-SLC26A3 from proteolytic digestion enzymes in the intestine [14]. The gene SLC26A3 has also extraintestinal expression: it is expressed in sweat glands, the male reproductive tract, and the kidney, while it is downregulated in human colon adenomas and adenocarcinomas [15–17].

The majority of the SLC26A3 mutations are single-nucleotide substitutions (nonsense, frameshift and missense), while additional mutations as deletions/insertions are minor. These mutations can interfere with the normal life of the protein in many ways: the protein may not leave the *Endoplasmic reticulum* (ER) or the traffic of the protein to the cell surface may be interrupted, as well as the activity of the protein may be reduced, absent, or alternated [13, 18]. There are no shreds of evidence of a correspondence between genotype and phenotype. It has been demonstrated that an identical genetic background of CLD may show different clinical courses [19] or different responses to therapy [20, 21].

Countries with the highest incidence are Finland (1:35,000), Poland (1:200,000), Kuwait (1 in 3200 births), and in Saudi Arabia (1 in 5500) [22–24]. In Arabian countries, the high incidence is due to consanguineous marriages [24]. There is a single mutation in the above-mentioned ethnic groups: in Finns, the p.V317del mutation affects up to 90% of CLD alleles; in Saudi Arabians and Kuwaitis, pG187X is present in more than 90% of altered chromosomes; in Poles, 50% of CLD alleles carry the p.1675dup [10].

The main clinical symptom is life-long watery urine like diarrhea with high Cl- content and low pH [1]. If not treated, diarrhea leads to dehydration, metabolic alkalosis, hyponatremia, hypokalemia, hypochloremia, and failure to thrive.

Usually, diarrhea causes metabolic acidosis; in CLD, a large volume of sodium-rich and bicarbonate low fluid is lost from the body, causing contraction alkalosis [25].

Because of the intrauterine onset of diarrhea, CLD pregnancies are characterized by bowel dilatation, polyhydramnios with normal peristalsis, and the newborn could be mild premature [24, 26]. High levels of amniotic Cl- have been described.

These features are sensible but not specific to CLD. Fetal MRI has been used to differentiate CLD/CSD from intestinal obstruction [7]. Postnatally, CLD patients present prominent abdominal distention due to dilated loops of ileum and colon filled with air and fluid: the loss of a big amount of fluid from the GI tract can lead to severe dehydration; ascites is often present. Hyperbilirubinemia is common and is

caused in part by the dehydration. It is worth remembering that excessive volume and salt depletion reduces the amount of diarrhea and may result in a low fecal Cl^- of even 40 mmol/L such as a stool dilution by urine [24, 27]. After the correction of electrolyte homeostasis by salt substitution, the first thing to do is the measurement of Cl- on a stool sample, eventually obtained with a soft catheter to avoid contamination with urine. A fecal Cl^- >90 mmol/L confirms the diagnosis in most of the cases and leads to molecular analysis of SLC26A3. Untreated patients show retarded growth and development, with mental and psychomotor retardation; a promptly start of therapy is associated with a better growth [19].

The main therapeutic approach is based on substitutive therapy with NaCl/KCl (see Table 36.1) [28]. The short-chain fatty acid, butyrate, is also beneficial in patients affected by CLD [21, 29]. Butyrate could reduce mistrafficking or misfolding of DRA and may enhance gene expression through the activation of a region crucial for high-level transcription. Butyrate is also able to modulate transepithelial ion transport through the stimulation of Na^+/H^+ exchangers 2 (NHE2) and 3 (NHE3) activity and inhibition of Cl- secretion by limiting the action of the cotransporter Na-K-2Cl (encoded by NKCC1) on enterocyte basolateral membrane. Treatment with oral butyrate (100 mg/kg/day) allows a progressive reduction to normal in the number of bowel movements and stool volume, an improvement in stool consistency, and a reduction of fecal incontinence episodes [20]. The starting dose is 50 mg/kg/die and a step-up approach is suggested to reach the 100/mg/kg/day. The effect of butyrate on the stool pattern is evident within the first 48 hours and remained stable during the following days of treatment. The response to butyrate may vary, and this explains the different benefits based on ethnicity [20]. A genotype-depending response was observed by some authors: patients with deletion and missense mutation showed a full response, while a partial response was observed in patients with nonsense or splicing mutations [20]. Other authors showed a different response in children with the same deletion mutation, suggesting that a more complex mechanism is responsible for the response [21].

The long-term prognosis of CLD patients is generally favorable. All of the patients treated adequately reach adult life, because oral salt substitution with NaCl and KCl allows normal growth and development, and the stool pattern tends to improve in the adult age [26, 28]. The most common complications are failure to thrive and psychomotor delay. Chronic kidney disease is described in up to 20% of CLD patients, and in some cases, dialysis or transplantation may be necessary [16]. In males, an important complication is subinfertility, caused by alteration of number, function, and morphology of sperms. In females, cases of pregnant women

Table 36.1 Therapeutic approach to a child with congenital chloride diarrhea

Treatment of acute dehydration			
1. Initial treatment of fluid depletion (over 6–8 h) IV infusion of 0.9% NaCl solution, NO fluids containing bicarbonate 120–500 mL/day for children under 7 years 500–1000 mL/day for children over 7 years			
2. Maintenance therapy (over 24 h) Intravenous 5% glucose 100 mL pro kg (children weight 10 kg) + 50 mL pro kg for each additional kg (children weight between 11 and 20 kg) + 20 mL pro kg for each additional kg (children weight 20 kg) For adult patients: 2000–2500 mL/day NaCl 20 mmol/L added KCl maintenance need + calculated K^+ depletion added (50 mmol/L) If severe hypokalemia, higher KCl doses of even 70 (−100) mmol/L			
3. Replacement of ongoing losses for diarrhea (0.9% NaCl and, if necessary, KCl)			
Salt substitutive therapy in stable clinical condition			
	Small children (0–3 years)	*Older children*	*Adolescents and adults*
NaCl	0.7% (7 g/L; 120 mmol/L)[a]	1.8% (18 g/L; 308 mmol/L)	Equal molar ratios of NaCl and KCl
KCl	0.3% (3 g/L; 40 mmol/L)[a]	1.9% (19 g/L; 255 mmol/L)	Equal molar ratios of NaCl and KCl
Concentration of Cl−	160 mmol/L	563 mmol/L	--
Need for Cl−	6–8 mmol/kg/day	3–4 mmol/kg/day	3–4 mmol/kg/day
Administration	Intravenous[b]/Oral	Oral	Oral
Doses per day	3–4	2–3	2–3
Pharmacological treatment			
Oral sodium butyrate, 100 mg/kg/day[c], divided in two doses			

[a]Intravenous therapy is used for infants until the shift to peroral fluid is possible. During intravenous therapy, the need for additional fluid is 100–120 mL/day (stool volume), which can be taken orally

[b]A "step-up" approach is recommended, starting from a dosage of 50 mg/Kg/day for 7 days, 75 mg/Kg/day for the next 7 days, reaching the final dosage of 100/mg/Kg after 14 days

[c]If a tendency to hyperkalemia: 0.9% NaCl (9 g/L; 154 mmol/L) and 0.2% KCl (2 g/L; 27 mmol/L)

with CCD have been described and ended up uneventfully. Additional complications include intestinal inflammation, hyperuricemia, inguinal hernias, and spermatoceles [26].

Congenital Sodium Diarrhea

Congenital sodium diarrhea (CSD; OMIM 270420) is a rare inherited disorder, characterized by severe diarrhea since birth, with metabolic acidosis, dehydration, and hyponatremia due to enormous fecal losses of Na^+. Fetal polyhydramnios, distended loops, and failure to pass meconium are common features of CSD [30]. Two variants have been described: syndromic CSD and classic CSD and three genes have been identified as relevant: SPINT2 for syndromic CSD, SLC9A3 and GUCY2C for classic CSD [31]. In 40% of classic type, no SLC9A3 or GUCY2C mutations have been found, suggesting that other genes may be implied [31] (Fig. 36.3). The syndromic form of CSD is an autosomal-recessive disease caused by loss-of-function mutations in SPINT2 and is characterized by choanal and/or anal atresia, hypertelorism, corneal erosions, double kidney, cleft palate, and digital anomalies [32]. Mucosal anomalies have been described, resulting in villous atrophy and characteristic tufts of extruding epithelium [31]. Mainly nonsense and splice-site mutation of SPINT 2 have been identified. SPINT2, also known as placental bikunin or hepatocyte growth factor activator inhibitor type 2 (HAI-2), is a potent inhibitor of several serine proteases, with particular attention to matripase and prostasin. These two serine proteases form a reciprocal zymogen activation complex that results in their autoactivation. In the distal colon, activated matriptase-prostasin complex is essential for proteolytic activation of ENaC, a channel is implied in Na + reabsorption. The absence of SPINT2

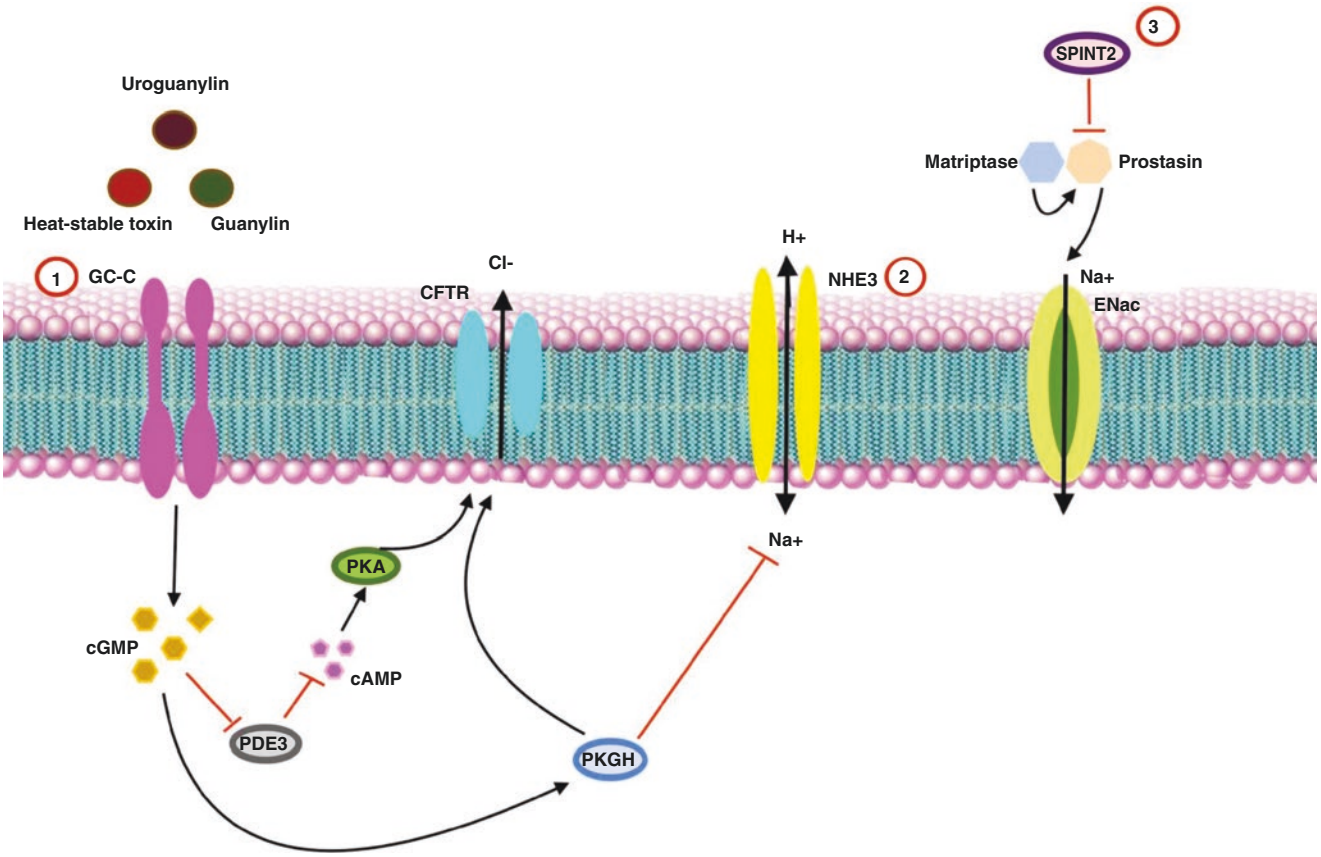


Fig. 36.3 Main pathophysiological mechanisms of congenital sodium diarrhea

1. Classic variant: The autosomal-dominant mutation of GUCY2C results in an alteration of the transmembrane guanylate cyclase GC-C; intracellular levels of cGMP rises without ligand binding. High levels of cGMP inhibit NHE3 via its phosphorylation by cGMP kinase II, causing a "secondary NH3 deficiency" and increase in Cl- secretion by CFTR via PKA

2. Classic variant: autosomal-recessive disease characterized by mutations in SLC9A3 gene that encodes for the NHE3, leading to a less absorption of Na + in the gut

3. Syndromic variant: autosomal-recessive disease caused by loss-of-function mutations in SPINT2, a potent inhibitor of matripase and prostasin. Activated matriptase-prostasin complex is essential for the proteolytic activation of ENaC, a channel that is implied in Na + reabsorption. The absence of SPINT2 results in a 'consumptive depletion" of matriptase, a secondary less activation of prostasin, and less proteolytic activation of ENaC with a decreased sodium absorption

results in a "consumptive depletion" of matriptase, a secondary less activation of prostasin, and less proteolytic activation of ENaC with a decreased sodium absorption [31].

Two forms of the classic variant have been reported. The first one is an autosomal-recessive disease characterized by mutations in SLC9A3 gene that encodes for the NHE3 channel. NHE3 is responsible for the major absorption of Na + in the gut and concurs in the acid-base homeostasis. Truncating and missense mutations were identified, resulting in a reduced function or expression of the transport [31]. The second variant is an autosomal-dominant mutation involving the GUCY2C gene that encodes for the transmembrane guanylate cyclase GC-C receptor for heat-stable enterotoxins that plays an important role in the pathogenesis of acute secretory diarrhea.

Uroguanylin, guanylin, and enterotoxigenic *Escherichia coli* activate this cyclase. The mutation of this gene results in its activation; high level of cGMP inhibits NHE3 via its phosphorylation by cGMP kinase II, causing a "secondary NH3 deficiency," and increases Cl- secretion by CFTR via PKA [31].

This gene is implicated also in familial diarrhea syndrome (FDS); however, the enhanced activity of GC-C in CSD is higher compared to FDS.

No mucosal anomalies have been described in classic type. Classic variant has been associated with a higher risk of inflammatory bowel diseases (IBD) [31, 33].

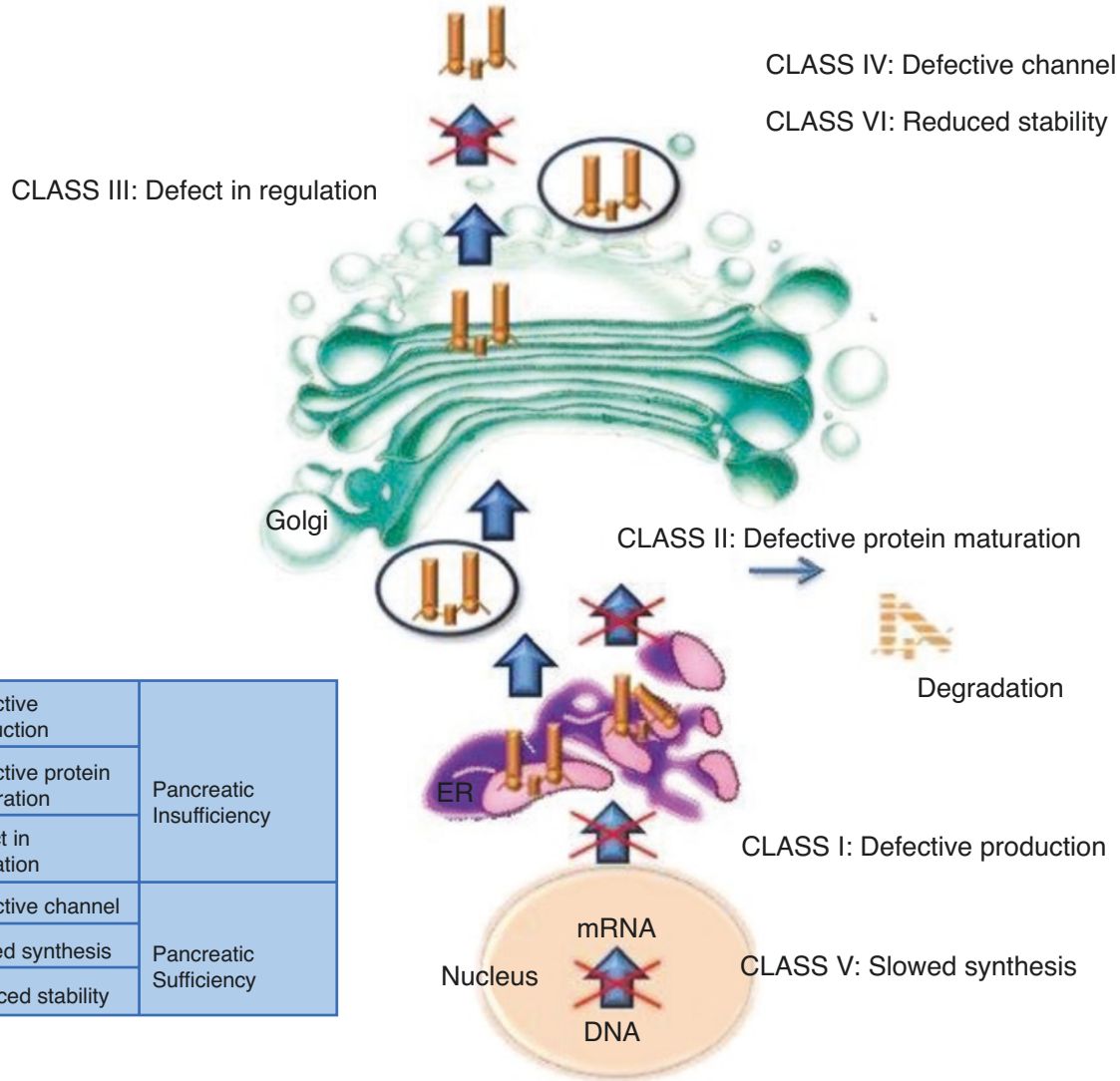
A diagnosis of CDS is made on the findings of life-threatening secretory diarrhea, with voluminous alkaline stools (pH >7.5), containing a high concentration of Na^+ (> 70 mmol/l) associated with hyponatremia (Na^+ <130 mmol/l) and metabolic acidosis. Fecal Na^+ could be normal after an excessive volume and salt depletion. CSD treatment consists of the replacement of losses ions and parenteral nutrition to acquire adequate caloric and fluid intake.

Familial Diarrhea Syndrome

This disease was described for the first time in 32 members of a Norwegian family [34]. Familial diarrhea syndrome (FDS) is characterized by early-onset chronic diarrhea, associated with meteorism, abdominal pain, dysmotility, and inflammatory bowel disease [34]. All affected members show a heterozygous missense mutation in *the GUCY2C* gene, c.2519G > T [p.Ser840Ile]. This gene is implied also in classic CSD [31]. It was been demonstrated that the basal GC-C enzyme activity, cellular cGMP levels, and affinities for ligands are similar in cells expressing either the wild receptor or the mutant one. In contrast, in CSD, basal GC-C enzyme activity is higher compared with wild type [33]. The mutant receptor in FDS is activated by heat-stable enterotoxin, uroguanylin, and guanylin to a greater extent than the wild receptor but not as high as the mutant receptor described in CSD [33]. A missense mutation leads to a gain of function, increasing ligand-mediated activation of GC-C and intracellular cGMP levels. GMP activates protein kinase GII, leading to phosphorylation of the CFTR channel [35]. This activation leads to an efflux of Cl^- (or HCO_3^- in the duodenum) and water into the intestinal lumen, with a reduced Na + absorption due to inhibition of the Na+/H+ exchanger 3 (NHE3) [36]. Another difference from CSD is the absence of prenatal anomalies: no polyhydramnios or bowel dilatation has been reported.

Cystic Fibrosis

Cystic fibrosis (CF; OMIM 219700) (CF) is an autosomal-recessive disorder due to a dysfunction of Cl- channel (CFTR) in the apical surface of epithelial cells. Because of the different locations of CFTR in the human body, CF is characterized by several and various symptoms and it is the main cause of exocrine pancreatic insufficiency in childhood. The prevalence of CF is 1/3500 and occurs with greater frequency in populations of North America, Northern Europe, Australia, and New Zealand [37]. The pathophysiologic basis of CF is characterized by a mutation of CFTR gene, located on chromosome 7, that determines the loss of function of CFTR (Fig. 36.4). CFTR is a member of the ATP-binding cassette (ABC) transporter superfamily. This membrane protein consists of two membrane-spanning domains, two nucleotide-binding domains (NBDs), and a regulatory domain, which controls channel activity, and it functions as a cAMP-dependent chloride channel. CFTR is largely expressed in epithelial cells of the airways, in the GI tract, including the pancreas and the biliary system. More than 2000 different mutations in the *CFTR* gene were identified; however, only 200 mutations cause the disease; the most frequent mutation, present in almost 70%, is the deletion of a single phenylalanine residue at amino acid 508 (ΔF508) [38, 39]. CFTR mutations have been divided into six classes based on how they interfere with the normal life of the protein; however, many mutations can be assigned in more than one class. Class I: No functional protein produced; Class II: Absent or diminished protein processing; Class III: Defective gating; Class IV: Decreasing conductance; Class V: Abnormal splicing; Class VI: Decreased surface stability [40]. Recently, Class VII has been introduced; it represents a variant of Class I. Class VII is also called "unrescuable mutation": no target therapy can be used and it is caused by mutations that result in absence of CFTR mRNA [41]. The loss of function of CFTR determines the inability to secrete NaCl in the respiratory tract. The small amount of water present on the surface of the mucous membrane is insufficient to hydrate secretions that become more viscous and elastic, resulting in



CLASS I	Defective production	Pancreatic Insufficiency
CLASS II	Defective protein maturation	
CLASS III	Defect in regulation	
CLASS IV	Defective channel	Pancreatic Sufficiency
CLASS V	Slowed synthesis	
CLASS VI	Reduced stability	

Fig. 36.4 Synthesis of CFTR and classes of mutation in cystic fibrosis
Synthesis of CFTR and classes of mutation in cystic fibrosis. CFTR gene encodes for a protein that regulates Cl – transport. CFTR is located at membrane level of secretive cells in lungs, gastrointestinal tract, liver, pancreas, and reproductive system. Mutation of Class I is related to the production of the protein: Generally, the protein is not produced at all or in very small quantities. Class II mutations prevent the maturation of protein. In Class III and IV mutations, protein is produced, but works incorrectly. Class V mutations allow a slow production of a small quantities of a working protein. Currently, the effects on clinical outcomes are not clear for all mutations, but a correlation between class mutation and pancreatic involvement is known: Class I, II, and III mutations are generally associated with pancreatic insufficiency, and Class IV and V mutations are associated with pancreatic sufficiency

a slowing of mucociliary clearance and airway obstruction. CFTR dysfunction can also make PH more acid in the respiratory tract. In the normal pancreas, the duct cells secrete Cl- via CFTR. Cl lumen level is important, because this ion will be exchanged with the intracellular HCO_3^-. The absence or dysfunction of CFTR results in a more viscous pancreatic secretion. On the contrary, since the function of sweat glands ductal cells is to absorb chloride, rather than secrete it, in CF, the concentration of chloride and salts in the sweat is very high. Primarily on this principle is based the most important CF diagnostic test. CF has a wide heterogeneity of clinical manifestations related to the high number of mutations and environmental factors. Pulmonary and GI symptoms are the most common. Chronic infections characterized the expression of CF in the airways and are determined by the inability to quickly eliminate microorganisms inhaled, resulting in colonization and inflammatory response in the airway walls. This phenomenon is also determined by the reduction of the secretions antimicrobial activity, resulting in excessive acidity of the fluid surface. Cough is the most common symptom

Table 36.2 Main clinical features of cystic fibrosis

Chronic respiratory disease
Persistent colonization/infection by typical pathogens *(Staphylococcus aureus, non-typeable Haemophilus influenzae, Pseudomonas aeruginosa, Burkholderia cepacia*
Chronic cough and sputum production
Persistent abnormalities on chest radiographs (bronchiectasis, atelectasis, infiltrates, hyperexpansion)
Airways obstruction characterized by bronchospasm and air trapping
Nasal polyps and sinus abnormalities evaluable at radiographs or CT
Digital clubbing
Gastrointestinal and nutritional involvement
Intestine: meconium ileus, distal intestinal obstruction syndrome (DIOS), rectal prolapse
Pancreas: pancreatic insufficiency, recurrent pancreatitis
Liver: chronic liver disease characterized by clinical or histological evidence of focal biliary cirrhosis or multilobular cirrhosis
Nutrition: protein–calorie malnutrition, hypoproteinemia, and edema, complications secondary to a deficiency of fat-soluble vitamins
Salt-wasting syndrome:
Acute salt depletion, chronic metabolic alkalosis
Urogenital abnormalities in male determining obstructive azoospermia

of pulmonary involvement, dry and hacking at first, then loose and productive, usually with purulent expectorated mucus [42]. GI involvement consists of two different clinical entities: meconium ileus and distal intestinal obstruction syndrome (DIOS), both due to an impaired electrolyte and fluid secretion that cause an inspissation of intestinal content and consequent intestinal obstruction, in combination with the known impaired intestinal motility. Classically, patients present fat maldigestion and malabsorption that can be directly attributable to the reduced secretion of lipase/colipase from the exocrine pancreas. Liver disease secondary to cholestasis is present in 30% of patients, and it is responsible for a variety of biliary tract and hepatic complications. Clinical manifestations of liver involvement are characterized by jaundice, ascites, hematemesis from esophageal varices, and signs of hypersplenism, hepatomegaly with steatosis [43]. Male sterility is a constant feature (Table 36.2). A correlation between mutation class and phenotype has been descried. Class I, II, III, VII mutations are associated with a severe phenotype, while Class IV and V results in a mild phenotype with pancreatic sufficiency. However, frequently, the mutations present on both alleles belong to different classes, and the clinical manifestations are the results of both. In addition, "CFTR-related disorders" have been described. In these cases, mutations on CFTR result in atypical disease, and manifestations are limited to few organs. These entities include episodes of recurrent pancreatitis or isolated bilateral bronchiectasis, bilateral agenesis of the vas deferens (CBAVD) with no digestive or respiratory involvement. The variability of clinical expression suggests that phenotype is not just the results of CFTR mutation but is also influenced by non-CFTR factors [44].

The diagnosis of CF is both clinical and genetic. A combination of a positive newborn screening, sweat test >60 and the identification of 2 CF-causing mutations (defined by CFTR2) with symptoms of CF, or positive family history is consistent with a diagnosis of CF. A monosymptomatic clinical entity (CBAVD/pancreatitis/bronchiectasis) associated with CFTR dysfunction that does not fulfill the diagnostic criteria for CF is defined as CFTR-related disorder.

Individuals who had a positive newborn screening but do not fulfill the diagnostic criteria of CF are defined: CFTR-related metabolic syndrome (CRMS) or CF screen positive, inconclusive diagnosis (CFSPID). They should receive a clinical evaluation by CF specialist in order to identify any possible clinical symptoms [45].

With newborn screening, diagnosis is often made before obvious clinical manifestations such as failure to thrive and chronic cough show up. Screening consists of a combination of immunoreactive trypsinogen results and limited DNA testing on blood spots, which are then coupled with confirmatory sweat analysis, and it is ≈95% sensitive [50].

Sweat test, which is considered the gold standard in the diagnosis of CF, is based on the determination of Na + and Cl- concentration in sweat collected after local stimulation with pilocarpine. Levels <35 mmol/L are considered normal; levels >60 mmol/L are considered pathological. Between 35 mmol/L and 60 mmol/L, results are considered borderline; in this scenario, sweat test must be repeated with an extensive CFTR gene mutation analysis, and this patient should receive a clinical evaluation by CF specialist. A positive sweat test alone does not allow to make a diagnosis and, on the other hand, it does not allow to exclude CF if negative: some mutations are associated with a negative sweat test [43, 46, 47]. Generally, sweat tests are not performed in subjects younger than 2 weeks of age and that weigh less than 3 kg. The test is contraindicated in babies younger than 48 hours of age, because high concentrations of sweat electrolytes can be found on the first day of life. If the patient is acutely unwell, dehydrated, edematous, or receiving corticosteroids, the test should be delayed [36]. Another important diagnostic test is the search for genetic mutations of the CFTR gene. Several commercial laboratories test for 30–96 of the most common CFTR mutations. These tests identify ≥90% of individuals who carry 2 CF mutations. These tests are used to confirm but not to exclude the dubious diagnosis, because a lot of mutations have not yet been identified. The genetic study can identify four different mutations categories: CF-causing mutation, mutation of varying clinical consequence, uncharacterized mutation, non-CF-causing mutation. In individuals with a positive newborn screen but with unchar-

acterized or mutation of varying clinical consequence CFTR mutations (<2 CF-causing mutations), the diagnosis of CF can be made by demonstrating CFTR dysfunction by a positive sweat test or nasal potential difference (NPD) or intestinal current measurement [45]. Among other possible diagnostic tests, the evaluation of potential differences across the nasal epithelium is often used. It consists of the demonstration of alterations in chloride secretion of epithelial cells, after stimulation with amiloride, through the measurement of nasal potentials. The assessment of pancreatic function is another test used in CF. This assessment is usually made through the evaluation of steatorrhea, fecal chymotrypsin, fecal elastase, and the duodenal assay after secretin or pancreozymin stimulation [48]. Also intestinal current measurements (ICMs) on rectal suction biopsies have been used to evaluate CFTR dysfunction [49]. The treatment of CF is extremely complex and articulate.

Respiratory system Therapy of bronchial obstruction is based on physiotherapy, which maintains adequate bronchial toilet and on aerosol therapy that has the purpose of liquifying secretions.

Anti-inflammatory therapy has given numerous benefits, despite the contraindications [51]. During exacerbations, an increase of physiotherapy in combination with antibiotic therapy is indicated. Antibiotic therapy should be guided by the results of sputum culture, preferably using intravenous drugs at high doses [52, 53]. In the most severe case, lung transplant is curative.

Gastrointestinal tract Pancreatic insufficiency is generally treated with substitution therapy by administration of pancreatic extracts formulated into microspheres gastroresistant. Therapy of meconium ileus and distal intestinal obstruction syndrome should be as conservative as possible, with the use of high volume water enemas and subsequently enema with gastrografin, in association with the rehydration therapy. Surgical therapy is reserved for cases of drug therapy failure. In addition, a hypercaloric diet is recommended.

Advanced in therapy New therapies have been approved and more are still in the experimental phase: correction of gene mutation, correction of the altered protein, and activation of the alternative channels to CFTR. Ivacaftor and Lumacaftor are the two mutation-specific drugs approved for clinical use. Ivacaftor is a potentiator and has been approved for Gly551Asp and eight other Class III mutations and for Class IV Arg117His mutation. Lumacaftor plus Ivacaftor is approved for the treatment of homozygous Phe508del [41].

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Congenital Disorders of Lipid Transport

37

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Introduction

Congenital disorders of intestinal lipid absorption include a clinically heterogeneous group of conditions whose molecular genetic basis has been assigned to key intracellular proteins that function in the delivery and transport phases of the lipid absorption pathway. These key intracellular proteins are encoded by gatekeeper genes that coordinate distinct components of a stepwise process involving uptake, compartmentalization, and complex intracellular lipid assembly, and their subsequent packaging into multicomponent vesicular structures called chylomicrons (CMs). The pivotal steps in CM assembly involve mobilization of membrane-associated complexes into vesicular transport vehicles within the lumen of the endoplasmic reticulum and Golgi, and ultimately vectorial delivery to the basolateral membrane of the enterocyte for extrusion and transport into mesenteric collecting lymphatics. The critical role of gatekeeper genes, particularly MTTP, APOB, and SAR1B, has been replicated and refined in both preclinical and cell-based models, and we now know more about the function and importance of their cognate proteins. Mutations, duplications, or deletions in these genes may give rise to steatorrhea with consequent fat malabsorption, failure to thrive, hypocholesterolemia, and hypotriglyceridemia in early childhood. In addition, unrecognized failure to thrive and lipid malabsorption syndromes may lead to progressive ataxic neuropathy and retinal degeneration because of the attendant malabsorption of fat-soluble vitamins, particularly vitamins A and E. With early diagnosis and appropriate nutritional supplementation, these particular complications can be avoided and general life expectancy should be unaltered.

We will review the major genetic syndromes associated with congenital lipid malabsorptions, the molecular genetic and pathophysiologic basis underlying defective lipid absorption, and describe the clinical manifestations and management of these patients.

Intestinal Lipid Absorption Overview

The reader is referred to recent comprehensive reviews of intestinal lipid absorption [1–4], but the essential features will be summarized below (Fig. 37.1). Complex lipids [principally long-chain TG, but also cholesterol ester (CE) and glycerophospholipids (GP)] are enzymatically lipolyzed to yield fatty acids (FA), cholesterol, and monoglycerides (MG), which are then transported through micelle-dependent uptake across the brush border membrane of villi, with distinctive membrane carriers responsible for FA and cholesterol uptake [3]. Once in the enterocyte, the lipolytic products (FA and MG) are bound to FA-binding proteins (FABP) and transported to the endoplasmic reticulum (ER) for synthesis of new TG, CE, and MG. The re-esterification of TG occurs mainly through the actions of diacylglycerol acyltransferase 1 (DGAT1), which likely functions in close proximity to the ER (Fig. 37.1). These TG droplets exist in both cytosolic (principally apical) compartments as well as within the ER membrane. Mobilization of TG droplets from within membranous domains requires the integrated actions of two gatekeeper genes for chylomicron (CM) formation: namely, microsomal triglyceride transfer protein (MTTP) and apolipoprotein B (APOB) with its intestine-specific isoform, APOB48. In this scenario, the resident endoluminal ER

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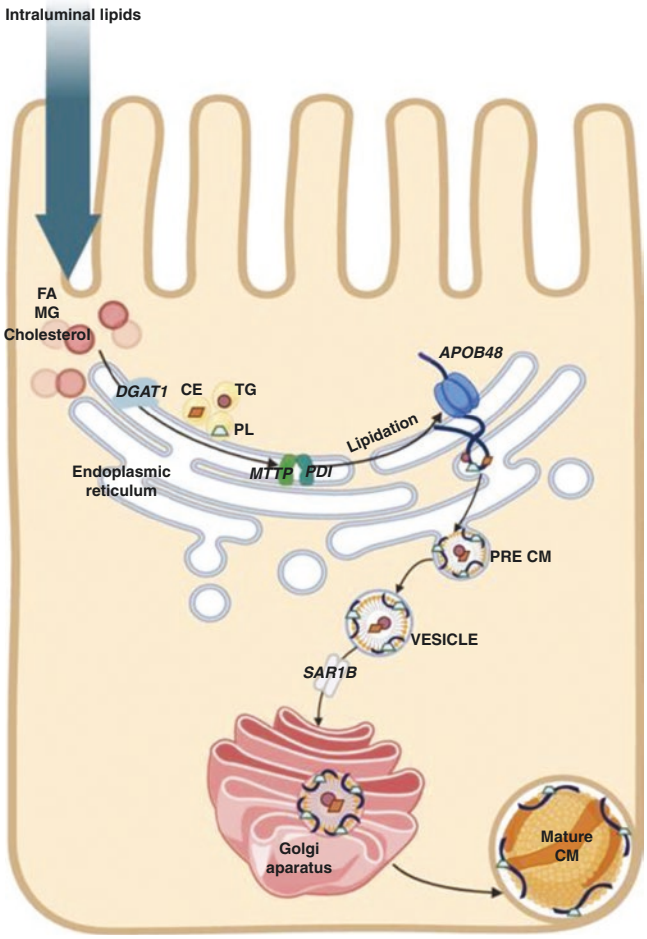

Fig. 37.1 Overview of intestinal lipid absorption: Key genetic regulators. Following digestion of dietary and biliary lipid, the component lipid species, including long-chain fatty acid, (FA) monoglycerides (MG), and free cholesterol (FC), are transported across the brush border membrane into the apical compartment of villus enterocytes. FA, MG, and FC undergo re-esterification into triglyceride (TG), cholesterol ester (CE), and phospholipid (PL), which is mediated in a series of enzymatic steps including diacylglycerol acyltransferase 1 (DGAT1). The newly synthesized TG exists in both cytosolic and intramembranous forms within the endoplasmic reticulum (ER) membrane, where DGAT1 also resides. The heterodimeric ER protein microsomal triglyceride transfer protein (MTTP) exists in a complex with the chaperone protein, protein disulfide isomerase (PDI), to physically translocate neutral lipid droplets from the ER bilayer membrane to an acceptor protein, APOB. Mammalian enterocytes synthesize a truncated APOB protein referred to as APOB48. The fusion of APOB and TG droplets, mediated by MTTP, is the critical and rate-limiting step in prechylomicron assembly. Following processing in the ER, the prechylomicron undergoes vesicular transport to the Golgi, mediated by fusion with COP proteins and SAR1B. Following further maturation in the Golgi, the chylomicron particle (CM), containing APOB48 and a cargo of TG, CE, and PL, is transported into the lymphatic compartment and delivered into the peripheral circulation

protein MTTP physically transfers the lipid droplet from the bilayer by mediating a physical interaction with the nascent amphipathic APOB protein, which acts as a structural acceptor during translation and extrusion into the ER lumen [5]. Luminal fusion of TG droplets with APOB48 generates a pre-CM particle, which then undergoes progressive modification and vesicular transport to the Golgi apparatus. This process is mediated by the Sar1-ADP Ribosylation factor, type B (SAR1B), which initiates vesicle formation for coat protein complex II (COPII)-dependent transport to the Golgi. The pre-CM particle then undergoes terminal modifications within the Golgi such as coat removal and acquisition of neutral lipids, and is ultimately secreted across the basolateral membrane as a mature CM (Fig. 37.1).

Molecular Genetic Basis for Congenital Defects in Lipid Absorption: Overview

Several rare genetic disorders of lipid absorption have been identified and their molecular basis characterized [5–9]. These include abetalipoproteinemia (ABL), which is an autosomal-recessive disorder caused by mutations or deletions in the *MTTP* gene [5]; familial hypobetalipoproteinemia (FHBL), an autosomal-codominant disorder, which is typically caused by mutations in the *APOB* gene [6]; chylomicron retention disease (CRD, also known as Anderson's disease), which is an autosomal-recessive disorder caused by mutations in *SAR1B* [7]; and congenital diarrhea, which is linked to defects in *DGAT1* [10, 11]. While the cardinal feature of lipid mucosal infiltration is common to all the disorders, the site of lipid droplet accumulation within the enterocyte shows subtle distinctions based on the molecular defects. Flaws associated with ABL and FHBL result in lipid droplet accumulation throughout the cytoplasm, both free and membrane-bound, but typically do not generate lipid particles within the ER lumen (Fig. 37.2a). Both ABL and FHBL interrupt intestinal lipid absorption at the initiation stage of pre-CM formation and thus completely attenuate CM assembly, which results in the generation of large intracellular cytosolic lipid droplets. As a result of the block in lipoprotein assembly and secretion (affecting both intestinal and hepatic lipoprotein secretion), the plasma in ABL and FHBL subjects contains very low levels of APOB containing lipoproteins (APOB48, APOB100) and virtually undetectable levels of very-low- and low-density lipoproteins (VLDL, LDL). Those subjects also manifest increased intestinal neutral lipid droplets containing TG and CE in the apical cytoplasm. These conditions will be discussed in more detail below. The defect in CRD, by contrast, is focused at a later stage in CM formation and blocks the maturation and exit of CM and pre-CM particles from the ER [12, 13]. The accumulation of lipid droplets and pre-CM particles within membranous domains is schematically illustrated in Fig. 37.2a. CRD will be discussed in a later section.

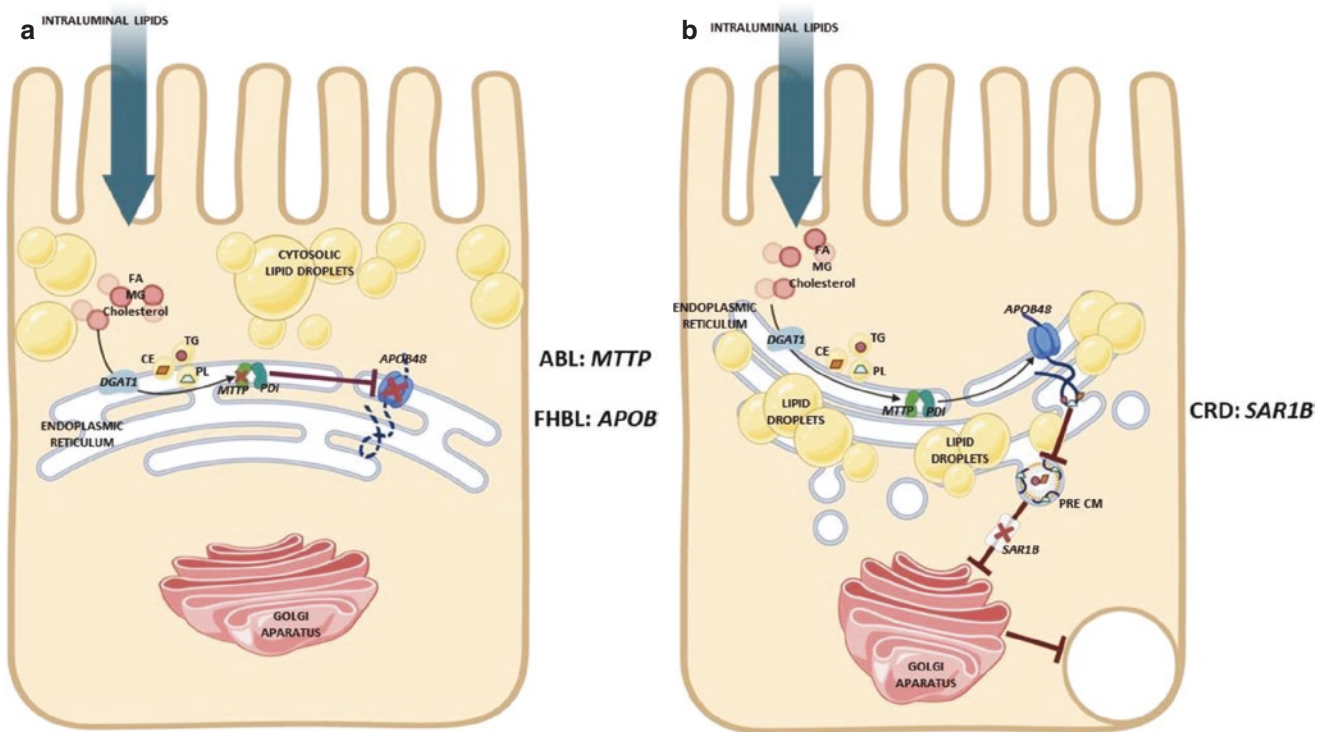

Fig. 37.2 (**a**, **b**) Lipid droplet accumulation in genetic disorders of intestinal lipid absorption. (**a**) Based upon the pathways outlined above, defects or mutations in *APOB* (responsible for familial hypobetalipoproteinemia, FHBL) or in *MTTP* (responsible for abetalipoproteinemia, ABL) eliminate the earliest steps in CM assembly and thus lead to lipid droplet accumulation, apical more than basolateral, with no lipid particles visible within ER profiles. (**b**) The phenotypes resulting from mutations or deletions in *SAR1B* (responsible for chylomicron retention disease, CRD) contrast with those for ABL and FHBL and result in interruption of the later stages of CM maturation and processing, leading to pre-CM accumulation (brown) within the ER lumen

Congenital Disorders of Chylomicron Assembly: Genetics and Clinical Features of Abetalipoproteinemia (ABL) and Familial Hypobetalipoproteinemia (FHBL)

Defects in either of the gatekeeper genes that promote CM assembly (i.e., *MTTP* and *APOB*) produce congenital intestinal lipid malabsorption. Both of these disorders generally present in infancy as failure to thrive, with (severe diarrhea), characteristically low serum lipid levels, and other complications (including ataxia and retinitis pigmentosa) along with the coincident malabsorption of fat-soluble vitamins [13]. However, the clinical phenotypes in both ABL and homozygous FHBL may be quite variable as discussed below.

Abetalipoproteinemia (ABL, OMIM #200100): Molecular Genetics and Prevalence

ABL is a rare, autosomal-recessive disorder with an estimated prevalence of <1 per 100,000 [14] and an estimated incidence of <1 per 1,000,000 [15]. To date, <30 MTTP mutations have been identified in more than 50 ABL patients (21.3% missense, 7% nonsense, 12% small insertion/deletion, 3% gross insertion/deletion, and 27.7% splicing variants) [15–22]. There is strong evidence for a founder effect in Ashkenazi Jewish patients, where a conserved haplotype with a common truncating mutation (p.G865X) was discovered in Israel with a carrier frequency of 1:131 and an estimated ABL incidence of 1:69,000 [23]. Some but not all of the reported ABL probands are the offspring of consanguineous marriage, an observation replicated across different national registries [18, 23]. A range of mutations and deletions has been described in the *MTTP* gene in association with ABL, and affected subjects may be either homozygous for a single mutation or compound heterozygous for different mutations. While there was initially thought to be no relationship between genetic heterogeneity and clinical phenotype, there is now some evidence to suggest differential severity based on the functional characterization of the individual mutations [24, 25]. Milder phenotypes have been associated with S5901 missense mutations [26]. In another report, two genetically distinct cases of ABL within the same family yielded starkly different phenotypes. The proband with concurrent MTTP mutations at p.R540C and c.1867 + 1G > A demonstrated only serum lipid derangements, while a cousin with a homozygous splicing mutation

at c.1867 + 1G > A had severe clinical manifestations that were refractory to high-dose vitamin supplementation [25]. Though other epigenetic and environmental factors could be playing a role, it appears that homozygosity results in a more severe phenotype than compound heterozygosity of the same mutation [25]. Certainly, more data is needed to better characterize the genotype–phenotype correlation in ABL.

Abetalipoproteinemia (ABL, OMIM #200100): Clinical Features and Management

Although ABL typically manifests in infancy as failure to thrive, there is a wide range of presentations in the first two decades of life, suggesting that there are either environmental or other genetic modifiers that influence some of the clinical features. Infants classically present with diarrhea, severe fat malabsorption, and failure to thrive. The hallmarks of ABL include extremely low plasma cholesterol and TG levels, very low or undetectable levels of APOB, low to absent levels of fat-soluble vitamins, essential fatty acid (EFA) deficiency, and red blood cell acanthacytosis [14, 17, 26].

The clinical phenotypes of low or absent levels of lipids and APOB in the serum result from the virtually complete block in lipoprotein secretion from both enterocytes and hepatocytes. This phenotype reflects the inability of both enterocytes and hepatocytes to lipidate the nascent APOB protein as it traverses the ER membrane (Fig. 37.2a).

The clinical features of ABL are multisystemic, including hematologic (acanthocytosis, anemia, hemolysis, coagulopathy), gastrointestinal (fat malabsorption, failure to thrive, hepatic steatosis, fibrosis, cirrhosis), neurodegenerative, musculoskeletal (myositis, muscle weakness, osteopenia), and cardiovascular (cardiomegaly, cardiomyopathy) [4]. Consequent to the fat malabsorption and defective CM production, patients have very low levels of fat-soluble vitamins, particularly vitamins A and E. Among the neurological sequelae of these vitamin deficiencies in ABL is pigmentary retinal degeneration. This condition can result in progressive vision impairment, starting with subtle losses of night and color vision in early stages and leading to complete vision loss if left untreated [4, 14]. Spinocerebellar ataxia is another devastating neurologic manifestation likely reflecting malabsorption of liposoluble vitamins [9]. The observation that ABL subjects, diagnosed and treated early with long-term high-dose supplementation of vitamins A (100–400 IU/kg/day) and E (100–300 IU/kg day) [27], maintain retinal and neurological function provides indirect but strong evidence that deficiency of these vitamins is responsible for the phenotypic outcomes [28, 29]. However, the exact mechanisms linking ABL to neurologic deficits are yet to be fully elucidated [29, 30].

ABL subjects require lifelong maintenance on a low-fat diet (<30% of total calories, 5–20 g/day) with reduced long-chain FAs to minimize steatorrhea [4, 28]. To avoid EFA deficiency, adequate intake of oils rich in polyunsaturated fats (i.e., olive, corn, safflower, soy bean; 5 g/day) should also be provided [4]. Early supplementation with oral fat-soluble vitamins is key. The current recommendations include vitamin A and E (as above), vitamin D (800–1200 IU/day), and vitamin K (5–35 mg/week) [4, 28]. For clinical monitoring purposes, it is worth noting that there is no discernible dose-to-tissue concentration relationship and that even with high-dose vitamin E supplementation, plasma levels rarely return to the normal range [31]. This is because of the dual defect that exists in vitamin E metabolism in ABL, reflecting both the block in intestinal absorption and also the inability of the alpha tocopherol transfer protein to repackage vitamin E into nascent VLDL in the liver [32]. Because circulating plasma lipid levels are so low in ABL subjects, plasma levels of alpha and gamma tocopherol may not reflect tissue (particularly adipose) concentrations, but nevertheless may be a useful surrogate to monitor long-term compliance [14]. Lastly, while there is conflicting data as to whether long-term vitamin A and E supplementation causes increased oxidative stress in the plasma of ABL patients, platelet antiaggregatory properties were never lost [29, 30, 33]. As such, the benefits greatly outweigh the risks.

ABL subjects warrant routine clinical monitoring to mitigate the severe complications associated with the disease process. Growth parameters are important markers of good nutritional status and should be closely followed. Although full growth potential might not be reached, with early intervention, ABL subjects can attain a normal growth velocity [28]. Additional long-term surveillance should include neurological and ophthalmological evaluations, bone mineral density scans, coagulation studies, and liver function tests and liver magnetic resonance spectroscopy to monitor for the development of steatohepatitis, which has been associated with progressive liver disease requiring liver transplantation [34, 35].

Familial Hypobetalipoproteinemia (FHBL, OMIM #107730): Molecular Genetics and Prevalence

FHBL is an autosomal-codominant disorder whose molecular basis most commonly resides in mutations in the *APOB* gene on chromosome locus 2p23-24 [36]. Unlike ABL, heterozygous FHBL subjects are typically asymptomatic with low circulating levels of total cholesterol, LDL cholesterol, and APOB generally discovered on routine lipid panels. Heterozygous FHBL, recognized through population-based plasma lipid screening for subjects at or below the 5th percen-

tile, is present in ~1:500–1:1,000 [36, 37]. The estimated birth prevalence of carrying a truncated APOB gene is 1:3,000 [38]. Homozygous FHBL (Ho-FHBL) by contrast is extremely rare with an incidence of <1:1,000,000 and perhaps 20 index cases whose molecular genetic basis is known [28, 39, 40]. The known mutations are linked to structural defects in the APOB gene, which interfere with the translation of the full-length protein APOB100 [4]. The truncated forms, ranging from APOB2 to ABOB89, are unable to undergo adequate lipidation despite normal levels of MTTP and thus prevent exportation of TGs via CMs from the enterocyte (Fig. 37.2a) and via VLDL from the liver [41]. The APOB fragments resulting from truncating mutations may be detected using denaturing gel electrophoresis and are present at very low circulating levels in plasma (undetectable if <27.6). Notably even heterozygous subjects exhibit lower abundance of the mutant (not exceeding 30%) compared to the wild-type (APOB100) allele [4]. This observation suggests the plasma lipid profile of the proband's parents may be an important diagnostic adjunct for delineating these conditions and further indicates that transcription or translation of the mutant allele in FHBL is also defective [42]. Serum lipid and APOB profiling can help delineate between Ho-FHBL and ABL in cases of diagnostic uncertainty. Namely, obligate heterozygote parents of Ho-FHBL will have markedly lowered total cholesterol, LDL, and APOB, whereas heterozygous ABL parents will be within normal range due to the recessive nature of the disease [28, 43].

In addition, FHBL is genetically heterogeneous with several kindreds described in whom the trait is inherited in an autosomal-dominant manner without mutations in the *APOB* gene but rather with linkage to a locus on chromosome 3p21, between D3S2407 and D3S1767 [42]. This low cholesterol syndrome linking to chromosome 3 has emerged as a unique entity, Familial Combined Hypolipidemia (FHBL2, OMIM #605019), caused by mutations in Angiopoietin-like protein-3 (ANGPTL3). ANGPTL3 is a hepatic-derived protein that inhibits lipoprotein lipase (LPL), the primary mechanism by which TG-rich lipoproteins are cleared, and endothelial lipase (EL) [41, 44]. Homozygous or compound heterozygous loss-of-function (LOF) mutations in ANGPTL3 are associated with increased lipolysis and clearance of lipoproteins with a subsequent decrease in circulating lipid levels [45]. FHBL2 is characterized by low plasma TC, TG, HDL, LDL, APOB, APOA1, and FFA without other clinical manifestations of FHBL (see below) [46]. Importantly, there appears to be no difference in the prevalence or severity of hepatic steatosis between FHBL2 subjects and noncarrier controls [41], and there is no intestinal lipid malabsorption phenotype. Data does suggest, however, that ANGPTL3 mutations confer a reduced risk of cardiovascular disease [44, 47]. The estimated prevalence of heterozygous carriers of LOF mutations in ANGPTL3 ranges from 1:309 to 1:237 [44, 48].

Mutations in proprotein convertase subtilisin/kexin type (PCSK9) are another well-described cause of FHBL. PCSK9 is a serine protease that augments degradation of hepatic LDL receptors to increase plasma LDL cholesterol levels. Loss-of-function (LOF) mutations cause dramatic increases in LDL uptake in the liver with consequent reduction in plasma LDL and APOB levels. Unlike APOB truncations, FHBL due to PSCK9 mutations is not associated with any clinical symptoms of fatty liver disease or intestinal lipid malabsorption. Conversely, PCSK9 LOF mutations also confer lower lifetime risk of cardiovascular disease in a gene dose–dependent manner [8, 41, 49].

Familial Hypobetalipoproteinemia (FHBL, OMIM #107730): Clinical Features and Management

The clinical manifestations of FHBL are heterogeneous. While heterozygous FHBL subjects are largely asymptomatic with mild fatty liver disease (3–5-fold greater than healthy controls), the phenotypic range for Ho-FHBL subjects is broad [40]. Severely affected individuals typically present in infancy or early childhood with failure to thrive, steatorrhea, and fat malabsorption, as in ABL. Yet others present in adulthood with incidentally discovered hepatic steatosis, very low levels (<5th percentile) of plasma lipids, and undetectable circulating APOB100 [4, 39]. This clinical variability has been linked to the degree of *APOB* truncation, with shorter isoforms (<34%) exhibiting an earlier-onset and more aggressive phenotype [35].

In general, the clinical course and management of severe Ho-FHBL subjects follows the same principles as described above for ABL. Homozygous cases demonstrate similar sequelae of intestinal fat malabsorption, including malnutrition, hypocholesterolemia, hepatic steatosis, acanthocytosis, and deficiencies in critical fat-soluble vitamins and essential FAs. If left untreated, these defects can result in progressive ocular, neuromuscular, hematological, and endocrine deficits with retinal degeneration, spinocerebellar ataxia, anemia, and insulin resistance, respectively [4, 28, 35, 50]. A more sinister concern longer term for FHBL subjects is the development of cirrhosis [51, 52] and hepatocellular carcinoma [9, 35, 39, 43]. The observations in relation to hepatic lipid accumulation in subjects with FHBL due to APOB truncations were not replicated in homozygous FHBL2 subjects linked to chromosome 3p21, reinforcing the concept that the clinical phenotypes among FHBL subjects are very variable [53, 54]. Homozygous FHBL subjects should therefore undergo regular evaluation of fat-soluble vitamin sufficiency with necessary supplementation and periodic monitoring of the myriad complications, as referenced above for ABL subjects. In this group, special attention should be given to the

potential for progressive hepatic steatosis and steatohepatitis (transaminases, liver magnetic resonance spectroscopy) and monitoring for progressive complications including fibrosis and hepatocellular cancer.

Chylomicron Retention Disease/Anderson's Disease (CRD, OMIM #246700): Molecular Genetics and Prevalence

Chylomicron retention disease/Anderson's disease was identified by Roy and colleagues in 1987 [55]. Although this disorder appears to be the same as that described by Anderson in 1961 [56], the term "CRD" is preferred nowadays, as it is more indicative of the pathology. CRD is a very rare, autosomal-recessive disorder with fewer than 50 cases described worldwide [12], and whose molecular genetic defect resides in the SAR1-ADP Ribosylation factor, type B (SAR1B), which belongs to the Ras superfamily of GTPases. SAR1B mutations induce an accumulation of pre-CM transport vesicles [12], as seen on intestinal biopsies and illustrated schematically in Fig. 37.2b.

SAR1B is a single polypeptide of 198 amino acids and functions as molecular switch controlled by the exchange of GDP and GTP. SAR1 is cytosolic in its GDP form and is recruited to the ER by the exchange of GDP for GTP to initiate COPII-coated vesicle formation. SAR1-GTP forms a coating protein complex (COPII) with two heterodimers SEC23/24 and SEC13/31. COPII initiates budding and captures cargo to eject vesicles from the ER to the Golgi apparatus [57]. The SAR1B GTPase cycle then allows cytosolic COPII proteins to exchange on and off the membrane at specific sites on the ER to regulate cargo exit. As a result, genetic defects in SAR1B affect the transport of pre-CM cargo from the ER to the Golgi apparatus where they usually undergo additional glycosylation before being released from the enterocyte. CMs are transported from the ER to Golgi, probably inside the pre-CM transport vesicle or PCTV [58]. PCTV shares several proteins including COPII (SEC13-31, SEC24, and SAR1) [59]. Siddiqi et al. [60] earlier showed that PCTV formation can occur in the absence of SAR1, and later showed that PCTV fusion with Golgi requires the presence of SAR1 [61].

The first investigation of genetic defects in eight families disclosed three frameshift (75-76 delTG, 555-558 dupTTAC, 349-1 G > C) and five missense mutations (109 G > A, 409 G > A, 537 T > A, 536 G > T, 542 T > C) [7]. The second exploration of the genetic abnormalities from eight families identified three new molecular aberrations: a stop codon mutation (364 G > T), a 5946 bp deletion (total exon 2), and a missense mutation (554 G > T) [62]. Thereafter, a new mutation G19T (changing a glutamic acid to a STOP codon) was found in the seventh exon [63], while two additional mutations were detected in exon 2 (G11D) and exon 4 (D75G) [64]. Recently, a novel homozygous deletion at position 142 (c.142delG) led to the replacement of the aspartic acid at position 48 by a threonine and gives rise to a frameshift-derived premature stop codon 17 amino acids further on (p.Asp48ThrfsX17), thereby resulting in a truncated protein (only 32% of the length of the normal protein and 24% of the normal sequence) [12]. Finally, the analysis of *SAR1B* gene mutations in Tunisian children revealed a proband homozygous for a novel nucleotide transition in exon 4 (c.184G > A), resulting in a nonconservative amino acid substitution (p.Glu62Lys) [65]. In order to provide a unifying explanation for the functional impairment of SAR1B mutated proteins, investigators have turned to information gleaned from the SAR1B crystal structure, computational analysis, and sequence alignment [66]. For example, the nonsense mutations and whole deletion of exon 2 in SAR1B yield truncated proteins that are predicted to modify the affinity of SAR1B for GDP and GTP, thereby affecting its interaction with the endoplasmic ER and other components of COPII machinery. In addition, the missense mutations in SAR1B likely yield nonfunctional proteins that alter the conformational and/or structural properties of SAR1B. Because SAR1B expression is detected in multiple different tissues, there are frequent clinical manifestations of CRD in organs beyond the gastrointestinal tract. As an example, cardiac abnormalities, myolysis, increased CK levels, and cardiac abnormalities were associated with the G19T mutation [63].

Genotype–Phenotype Associations in CRD

Recent studies compared the patients of two cohorts of CRD patients from France and Canada in order to compare the severity and long-term evolution as a function of the SAR1B gene mutations [67]. In both series, most of the CRD patients were symptomatic at diagnosis (before age of 1) and the disease was definitely more severe in infants. This is consistent with the observation that the CRD had profound impact on weight *Z* scores in Canadian children (1.5 ± 1 years) who were significantly younger at diagnosis than the French subjects (6.4 ± 1.3 years). The catch-up growth and bone mineral density improvement in the French cohort were not as encouraging in the French cohort compared to the Canadian patients, possibly because of the delayed initiation of a low-fat diet with a high polyunsaturated/saturated fatty acid ratio and of supplements of fat-soluble vitamins A, D, and E. In general, the neuro-ophthalmic manifestations detected at the time of diagnosis proved largely reversible with treatment. Although the effect of missense mutations on the protein was considered less deleterious than those mutations that introduce stop codons or that specified deletions, the clinical phenotype (i.e., growth, neurological impairment, hepatic

steatosis, lipid disturbances) was not significantly different in CRD patients based on the underlying genetic mutation [67]. According to the findings of this largest group of CRD patients so far reported, genotype–phenotype correlations are not obvious, suggesting that CRD might represent a more complex trait rather than a simple autosomal-recessive disorder [67]. Additional work is required to determine whether modifier genes are involved in the ER-to-Golgi transport.

Chylomicron Retention Disease/Anderson's Disease (CRD, OMIM #246700): Clinical Features and Management

CRD presents with failure to thrive probably resulting from fat malabsorption and constant, nonspecific diarrhea that begins shortly after birth. Other digestive symptoms include vomiting or abdominal swelling. Light microscopic examination generally reveals few morphological irregularities in the small intestine of CRD patients [12]. Following an oral fat bolus test meal and ultrastructural examination, numerous CM-size lipid droplets are evident within the cytoplasm of enterocytes. Many of these CM-size lipid droplets are bound by membranes of the smooth ER and clustered mostly in the supranuclear region, whereas only few of them were visualized within vesicular structures, likely part of the Golgi complex. The larger lipid vacuoles are not membrane-bound [13]. Importantly, the intercellular spaces were completely juxtaposed and no CMs were evident in the interstitium between adjacent enterocytes and in the area around the basement membrane. Hepatomegaly and a moderate degree of macrovesicular steatosis have been detected in a few cases [55, 68]. Nonspecific moderate hepatic cytolysis is very frequent but correlates only poorly with steatosis and/or hepatomegaly. Areflexia (combined with proprioceptive abnormalities) and electromyographic abnormalities (reduction in sensory nerve conduction velocity and decreased sensory nerve action potential amplitudes) were among the neurological abnormalities described in CRD children [55, 69, 70], but more severe neurological degeneration, such as ataxia, myopathy, and sensory neuropathy, has also been reported in CRD adults [71]. Sometimes, muscular pain and cramps are present in CRD children, while cardiomyopathy with a decrease in ejection fraction to 40% (normal >60%) has been described in adults [63]. Only minimal ophthalmic complications characterize CRD patients and include micronystagmus, mild deficit in the perception of the blue-yellow axis, and delayed dark adaptation [55], while abnormal visual evoked potentials have been evidenced by functional testing [72]. Poor mineralization and delayed bone maturation have also been observed in CRD [69].

Fat malabsorption in CRD impairs the absorption of fat-soluble vitamins, with vitamin E being the most affected, as observed in ABL subjects [73]. Vitamin A levels in plasma are frequently decreased, but generally correctable following oral supplementation. Finally, vitamin D or K insufficiency can be detected in up to half of CRD patients, and can also be corrected with oral vitamin supplementation. In CRD, acanthocytosis is rare, sometimes transient. Acanthocytosis when it occurs reflects low plasma vitamin E levels, which is a key to red cell membrane integrity. In association with muscular abnormalities, creatine kinase levels were elevated at diagnosis and have been associated with neurological impairment [72]. As previously noted, hepatic abnormalities may be present.

In contrast to ABL and FHBL, fasting triacylglycerol concentrations are usually normal, but severe hypocholesterolemia is prominent with levels of low-density lipoprotein-cholesterol and APOB100 at the lower limits of normal. Similarly, CRD patients display limited capacity to produce high-density lipoproteins. Importantly, CMs failed to appear in the circulation after a fat meal test explaining the deficiency of fat-soluble vitamins and essential fatty acids. However, patients with CRD express the apolipoprotein species essential for CM synthesis, including APO-AI, APO-AIV, and, importantly, APOB48 [12, 74].

Although CRD resembles ABL and FHBL symptomatically with respect to dietary fat malabsorption and its consequences, examination of plasma reveals a key distinction. Plasma from CRD patients contains APOB100 at normal levels but no APOB48 especially after a fat meal. As detailed above, plasma from ABL and FHBL subjects contains virtually no detectable APOB100, because the defect impairs secretion of lipoproteins from both the liver and small intestine [14]. Intestinal transcription of APOB100 mRNA and its posttranscriptional editing to APOB48 mRNA was normal in CRD subjects [75]. Both APOB and MTTP along with CM-size lipid droplets were detected in enterocytes from CRD subjects, suggesting that enterocytes can synthesize prechylomicron particles, but fail to process these intravesicular particles into mature chylomicron particles that can be secreted into the lymphatic circulation. Other attempts to identify the underlying mechanisms in CRD confirmed that the primary defect is not in the de novo synthesis of APOB48 protein, although reduced glycosylation of APOB was revealed in intestinal biopsies taken from CRD patients [74].

As with ABL and homozygous FHBL, CRD needs to be recognized early because of its adverse effects on growth and potential for neurological, ocular, hepatic, and other extraintestinal complications. Accurate diagnosis can be readily established using ultrastructural identification of CM-size lipid droplets clustered in the enterocytes, with the absence of fat outside the cells. However, sequencing of the short SARA2 gene provides a quick, safe, nonexpensive, and noninvasive diagnostic tool. Consumption of medium-chain triglycerides can aid in more rapidly correcting malnutrition in

infants, but total fat intake and particularly long-chain fatty acid consumption should be limited. The patients must be advised to take adequate intake of essential fatty acids and fat-soluble vitamins. In addition, vitamin E supplementation is critical for the prevention of the progression of neurological findings, while vitamin A should be administered at high doses to prevent ophthalmologic complications. Early vitamin D supplementation is also recommended in all patients to avoid bone growth and development abnormalities, and vitamin K should be given in cases of coagulopathies and abnormal clotting parameters.

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Immunodeficiency Disorders Resulting in Malabsorption

38

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Introduction

Primary immunodeficiencies are a group of at least 200 disorders, often inherited, that are caused by intrinsic defects of the immune system. The defects can affect humoral (B cell) immunity, cellular (T cell) immunity, both T and B cell immunity, and innate immunity. The adaptive and innate immune cells are generated and produced in the primary lymphoid organs (bone marrow and thymus), while the coordination of adaptive immune response takes place in the peripheral secondary lymphoid organs (spleen, lymph nodes system, and epithelial- and mucosa-associated lymphoid tissues such as Peyer patches in the small intestine) [1].

Gut is considered the largest lymphoid organ in the body, containing the majority of lymphocytes and producing large amounts of immunoglobulins (Ig). Gastrointestinal (GI) disorders affect about 5–50% of patients with primary immunodeficiencies, and severe malnutrition could be present in 50–60% of cases. The mucosal immune system, together with gut microbiome, plays a pivotal role in host defense against infections and in maintaining immune tolerance through direct and indirect mechanisms. In particular, gut microbiome could influence host immune system by the production of immunoregulatory metabolites such as short-chain fatty acids (SCFAs). Dysfunction of the regulatory mechanisms maintaining this complex and dynamic balance in the gut may lead to damage and mucosal inflammation and development of GI diseases [1–3].

It is not surprising that GI disorders, besides being very common are often the initial presenting manifestations in patients with primary immunodeficiencies (Table 38.1) [3].

In pediatric patients, GI manifestations of primary immunodeficiencies are mainly induced by infection, inflammation, or autoimmunity. In adult patients, malignancy could be a frequent additional condition. These manifestations, showing the same clinical features, could mimic the classic forms of diseases (in the absence of immunodeficiency) such as celiac disease, food allergy, or inflammatory bowel disease, but they are often unresponsive to conventional therapies.

Evaluating a Child with Suspected Primary Immunodeficiency

GI disease may be the first presentation of an underlying immunodeficiency; for this reason, it is crucial to consider immunodeficiency in any child with recurrent or chronic severe diarrhea, malabsorption, and failure to thrive that is resistant to conventional treatments. In the diagnostic investigation of chronic diarrhea, primary immune deficits (PIDs) should be included. The European Society of Immunodeficiencies (ESID) has suggested 10 warning signs for suspicion of PID (Table 38.2) [2].

Primary immune deficits are relatively common (1:500) but likely underdiagnosed [1].

The type of immunodeficiency provides susceptibility to different infectious agents: prevalence of bacterial infections could indicate B cell deficiencies mostly if they occur after 6 months of life, since maternal Igs protect the newborns

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Table 38.1 Main primary immunodeficiencies and associated gastrointestinal features

Immunodeficiency	Main gastrointestinal manifestations
Selective IgA deficiency	Chronic diarrhea Celiac disease Nodular lymphoid hyperplasia
Agammaglobulinemia, X-linked or AR	Chronic diarrhea Malabsorption IBD-like disease
Hyper-IgM syndrome	Chronic diarrhea Progressive liver disease Sclerosing cholangitis Liver cancer
Common variable immunodeficiency	Chronic diarrhea Nodular lymphoid hyperplasia Flat villous lesions IBD-like disease Atrophic gastritis
Severe combined immunodeficiency	Chronic diarrhea Oral candidiasis IBD-like disease
Chronic granulomatous disease	Granulomatous colitis Perianal fistulae Hepatic abscess Gastric outlet obstruction Small bowel obstruction Granulomatous stomatitis Oral ulcers Esophageal dysmotility IBD-like disease
Wiskott-Aldrich syndrome	Colitis Malabsorption IBD-like disease
IPEX and IPEX-related disorders	Severe enteropathy with watery often bloody diarrhea associated with eosinophilic inflammation
Interleukin-10 and interleukin-10-receptor defects	IBD-like disease with early-onset enterocolitis, perianal disease (multiple abscesses and enterocutaneous fistula)
Hermansky–Pudlak syndrome type 1	Granulomatous colitis

IBD inflammatory bowel disease, *IPEX* immune dysregulation, polyendocrinopathy, enteropathy, X linked [3]

Table 38.2 Warning signs for suspicion of PID

≥4 new ear infections within 1 year
≥2 serious sinus infections within 1 year
≥2 pneumonia infections within 1 year
Failure of an infant to grow normally or gain weight
Recurrent abscesses of deep skin or organs
Persistent oral or skin thrush
Requirement for intravenous antibiotics to resolve infections
≥2 deep-seated infections
A family history of PID

PID primary immune deficits [2]

during this period (Fig. 38.1). Fungal or viral infections, mostly if they occur at birth or within 6 months of life, could indicate T cell deficiencies, while severe mixed (bacterial, viral, and/or fungal) infections, particularly early in life, suggest combined (T and B) immunodeficiencies.

Careful clinical evaluation is crucial for recognition of patients with PID. A full physical clinical exam is mandatory: facial anomalies, absence of lymph nodes, and atypical heart murmur suggest DiGeorge syndrome; absence of lymph nodes with physiological heart sounds are classic features of severe combined immunodeficiency (SCID). Also skin manifestations (such as eczematous dermatitis, chronic mucocutaneous candidiasis, and cutaneous viral infections such as disseminated molluscum contagiosum) may be a clinical presentation of PID [4].

Due to the high incidence of GI disease in these patients, early evaluation of the GI tract is useful for children with certain or suspect immunodeficiency, to prevent potentially irreversible tissue damage [3].

Diagnostic algorithm in child with recurrent GI symptoms needs for a stage approach, in order to rule out differential diagnosis, to perform screening immunological tests, and to refer an immunologist when an immunodeficiency is suspected (Table 38.3) [3, 5, 6].

An accurate microbiological analysis of stool samples is mandatory to rule out the presence of common or unusual pathogens (e.g., *Giardia lamblia*).

Immunological screening tests include complete blood count, evaluation of quantitative levels of immunoglobulins (IgG, IgA, IgM, and IgE), and antibody titers. Hypogammaglobulinemia can result from impaired production and/or protein loss; the latter is excluded by measuring serum albumin and urinary protein levels; enteral loss of protein can be excluded by measurement of stool alpha-1-antitrypsin level (normal values <0.9 mg/g stool) [1, 7].

Based on medical history, clinical manifestations, and pattern of infection, if humoral defect is suspected, quantification of IgG subclasses may be helpful in the assessment of an immunodeficiency, especially in IgA deficiency. Ig serum levels increase during the life course, so comparison with age-matched controls is necessary for correct interpretation. Further evaluation of a humoral defect includes the qualitative aspect of the antibody response, such as IgG titer to measles, tetanus, *Haemophilus influenzae* type b, pneumococcus, and varicella. If antibody titers are absent or low, vaccinations may be administered, followed by evaluation of postvaccination titers 4–6 weeks later. It is important to underline that children receiving chronic treatment with steroids may have reduced serum Ig levels; however, the antibody response would be preserved in this case [1, 7].

When mixed and/or T cell deficiency are suspected, especially in the presence of blood cells count abnormalities, it is important to ask for a lymphocyte panel to assess the number of lymphocytes and subpopulations (T cells, B cells, CD4+ T cells, and CD8+ T cells), because lymphopenia may occur secondary to excessive loss of the cells into the lumen or through the trapping of cells in the inflamed bowel wall. Severe lymphopenia in an infant (<2000/mm^3) is a critical finding that, if associated with history of recurrent and severe

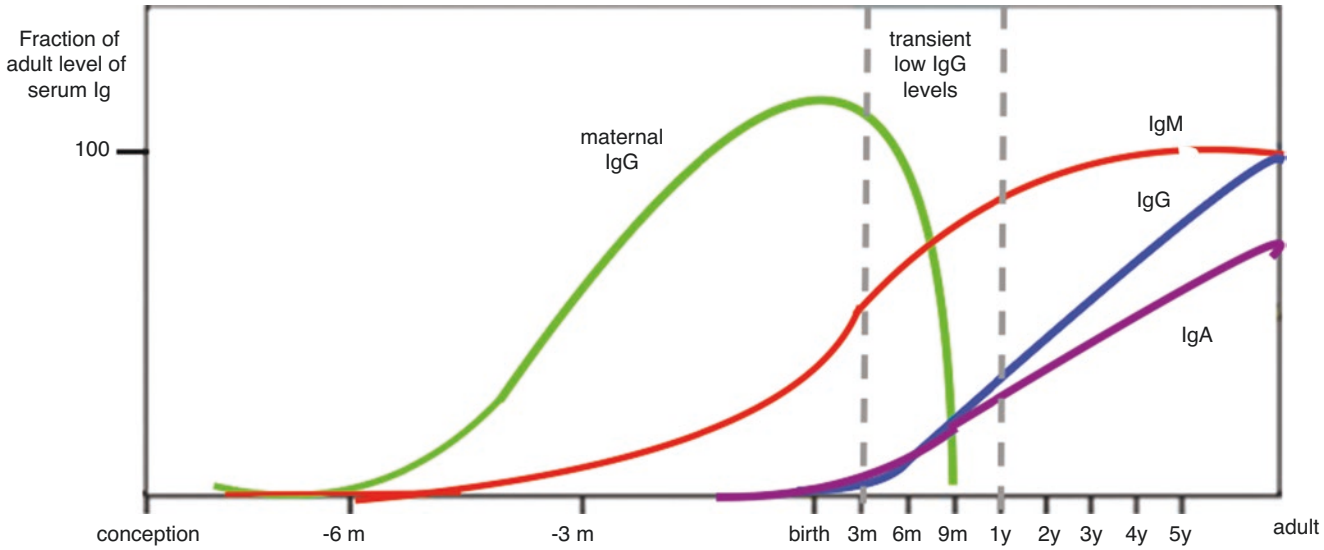

Fig. 38.1 Immunoglobulin (Ig) serum levels from conception to adult life
m month, *y* year
For the first 6 months of life, maternal IgG, that passively crosses the placenta during gestation, is mainly responsible for the immune protection of the newborn. In the newborn, Igs start to be produced around 6 months of life and they gradually increase during the life. The period between 3 and 12 months could be characterized by transient low IgG levels due to maternal IgG reduction and gradually infants IgG production [1, 2]

Table 38.3 Differential diagnosis in children with GI symptoms

Food allergy
GI infections
Antibiotic-associated diarrhea
Congenital diarrheal disorders
Celiac disease
Inflammatory bowel disease
Metabolic disorders
Cystic fibrosis
Primary immune deficit
Irritable bowel syndrome

Refs. [3, 5, 6]

Table 38.4 Secondary immunodeficiencies

Causes of secondary immunodeficiencies	Examples
Drugs	Anti-inflammatory Immunomodulatory Immunosuppressive Cytotoxic agents Anticonvulsants
Genetic diseases	Trisomy 21 Cystic fibrosis
Infectious diseases	HIV Epstein–Barr virus Cytomegalovirus Parvovius B19 Congenital rubella
Radiation	
Malignancies	Chronic lymphocytic leukemia Good's syndrome Hodgkin and non-Hodgkin lymphoma
Surgery and trauma	
Immunoglobulin loss	Protein losing enteropathy Nephrotic syndrome Plasma exchange Severe dermatitis

Refs. [8, 9]

infections, should lead to an immediate immune evaluation for severe combined immunodeficiency (SCID). Thrombocytopenia and small platelet size, mostly if eczema and recurrent bacterial infections occur, suggest a diagnosis of Wiskott–Aldrich syndrome (WAS). In all cases, exclusion of secondary causes of immunodeficiencies should be performed, mostly if there is clinical suspicion based on history or results of the lymphocyte panel (Table 38.4) [8, 9].

Depending on the clinical picture and on the results of these initial tests, the further step is the study of cellular immune function. The study of lymphocyte proliferation in response to mitogens and antigens provides more definitive data on T cell function. Failure of lymphocytes to respond to mitogens usually indicates severe impairment of T cell function, as in the case of SCID. These tests may not be possible in patients with significant T cell lymphopenia. Advanced testing can be performed to investigate specific disorders. For example, when chronic granulomatous disease (CGD) is suspected, investigation of neutrophil function is mandatory; this is accomplished with a dihydrorhodamine assay to determine a reduction or absence of phagocytic respiratory burst. Flow cytometric analysis of expression of cell surface and intracellular proteins can help in immune deficiency differential diagnosis: X-linked hyper-IgM (through the examination of CD40 ligand), WAS (through the examination of WAS protein, WASp), and immune dysfunction, polyendocrinopathy, enteropathy, and X-linked syndrome (IPEX; through the examination of FOXP3). Quantitative, real-

time polymerase chain reaction for T cell receptor excision circles is used in the neonatal screening assay for SCID. Genetic testing to identify carrier states or specific mutations can be performed for X-linked agammaglobulinemia (XLA), SCID, and DiGeorge syndrome (Table 38.5). In many cases, the evaluation of GI tract, in addition to laboratory tests, can be done via radiographic imaging and intestinal biopsy specimens. It is often helpful to ask the pathologist to review slides when a question of immunodeficiency exists, given some of the unique pathologic findings or lack thereof (e.g., plasma cells) [3].

Table 38.5 Primary immunodeficiencies: main laboratory findings and molecular defects

Immunodeficiency	Laboratory findings	Molecular defect
Selective IgA deficiency	Serum IgA absent or near absent, usually <10 mg/dL Normal IgG and IgM levels although IgG2 subclass deficiency may be present Impaired specific antibody response in some patients	Gene defect unknown Defective maturation of B cells into IgA-secreting plasma cells
Agammaglobulinemia, X-linked or AR	Absent IgM, IgG, and IgA B cells <1% of lymphocytes Absent specific antibody response	X-linked (BTK) Autosomal-recessive (μ heavy chain, λ5, Igα, Igβ, BLNK, PIK3R1, LRRC8, and TCF3)
Hyper-IgM syndrome	Low IgG and IgA Normal or increased IgM Normal or increased B-cell numbers Impaired specific antibody response Decreased T-cell responses in CD40L/CD40 deficiency	Mutations in CD40L, CD40, AICDA, UNG, NEMO, IKBA, IκBα, PI3K
Common variable immunodeficiency	Low IgG and IgA and/or IgM Absent specific antibody response Normal or decreased B-cell numbers Variably decreased T-cell responses	Mutations in ICOS, CD19, mutations in ICOS, CD19, CD20, CD81, TNFRSF13B, TNFRSF13C; mostly unknown
Severe combined immunodeficiency	Low TRECs Decreased serum immunoglobulins Marked diminished/absent T-cell, B-cell, and NK cell numbers depending on functional deficiency Diminished response to mitogens PHA, ConA, PWM	Multiple defects: RAG1/2, JAK3, CD45, CD3 chain, ZAP70, Artemis, ligase 4, Cernunnos, IL-2RG, IL-7Rα, ADA; defects in T and B cells
Chronic granulomatous disease	Defective oxidative burst in neutrophils by DHR or NBT equivalent	Multiple defects: X linked owing to defects in CYBB encoding the gp91 phox component of NADPH oxidase autosomal recessive owing to defects NCF1, NCF2, or CYBA defects in components of NADPH oxidase
Interleukin-10 and interleukin-10-receptor defects	Pathological response to functional tests using STAT3 and/or TNF-α assays	Mutations in IL-10, IL-10 receptor
Wiskott-Aldrich syndrome (WAS)	Immunoglobulins variable in concentration secondary to accelerated synthesis and catabolism (decreased IgM; normal or slightly low IgG; often increased IgA and IgE) Antibody response to polysaccharides decreased. Normal B-cell numbers. Progressive decrease in T-cell numbers with abnormal lymphocyte responses to anti-CD3. Platelet numbers are reduced and small in size.	Mutations in WAS; cytoskeletal defect affecting hematopoietic stem cell derivatives
Hermansky–Pudlak syndrome, type 1	Normal platelet count Prolonged bleeding time with abnormal platelet function assays Absence of δ granules on freshly isolated plasma with electron microscopy	Mutation in the HPS1 gene on chromosome 10q23 that forms part of BLOC-3, HPS2, HPS3, HPS4, HPS5, HPS6, HPS7, HPS8, HPS9, HPS10

ADA adenosine deaminase, *AICDA* activation-induced cytidine deaminase, *AR* autosomal recessive, *BLNK* B cell linker protein, *BLOC-3* biogenesis of lysosome-related organelles complex-3, *BTK* Bruton tyrosine kinase, *CYBA* cytochrome b α subunit, *CYBB* cytochrome b β subunit, *DHR* dihydrorhodamine, *ICOS* inducible costimulator, *JAK3* Janus activating kinase 3, *NADPH* nicotinamide adenine dinucleotide phosphate, *NCF* neutrophil cytosolic factor, *NF-κB* nuclear factor-kB, *PHA* phytohemagglutinin, *PWM* pokeweed mitogen, *RAG* recombinase activating gene, *TBX1* T-box 1, *TNFRSF* TNF-receptor superfamily, *UNG* uracil DNA glycosylase, *STAT3* signal transducer and activator of transcription-3, *TNF-α* tumor necrosis factor-α, *NBT* nitroblue tetrazolium, *NEMO* nuclear factor-kappa B essential modulator, *PIK3R1* phosphoinositide-3-kinase regulatory subunit 1, *LRRC8A* leucine-rich repeat-containing protein 8A, *TCF3* transcription factor 3 [7, 11, 17–19, 25, 26, 30, 31, 36, 38, 41, 45–49, 52, 59, 61].

Predominant B-Cell (Antibody) Deficiency

Selective IgA Deficiency

Selective IgA deficiency is the most common primary immunodeficiency; it is defined as a decreased serum level of IgA (IgA <7 mg/dL) in the presence of other normal Ig isotypes, T-cell immunity, and natural killer activity. The worldwide incidence of IgA deficiency differs by ethnic background; the prevalence varies among ethnicities from 1:100 to 1:1000, in Caucasians being much higher than that in Asians, with a prevalence of around 1:3000 in the USA [10].

IgA is the key Ig in the respiratory and GI tracts, which provide the most intimate interface between the environment and self [11]. The reduced, and not absent, counts of IgA-bearing B cells in the peripheral circulation and the absence of serum IgA suggest that this disease is the result of a defect in IgA-bearing B lymphocyte to turn into IgA-secreting plasma cells [12].

The manifestations are variable in IgA deficiency; patients can be asymptomatic (in up to 90% of subjects) or can incur in recurrent infections of the respiratory (particularly *H. influenzae* and *Streptococcus pneumoniae*) and GI tracts, autoimmune diseases, allergies, and malignancies.

Autoimmune diseases are a frequent finding in IgA deficiency, mainly autoimmune cytopenias, juvenile rheumatoid arthritis, thyroiditis, systemic lupus erythematosus, and celiac disease.

IgA deficiency sometimes progresses to common variable immunodeficiency (CVID); in fact, these diseases often share a common genetic and familial predisposition (IGAD1) [10].

Other related conditions include anaphylactic transfusion reactions; if replacement therapy is required, the patients should be screened for anti-IgA antibodies and treated with low or absent IgA blood products if the need for transfusion arises.

GI manifestations are frequent in symptomatic patients with selective IgA deficiency. *Giardia lamblia* infections can occur in these patients, causing bloating, cramping, excessive flatus, and watery diarrhea. The infection can be chronic, despite treatment with metronidazole, resulting in malabsorption with steatorrhea and villus flattening [13]. The degree of mucosal damage is related to the duration of the infection. Diagnosis is made by examining the stool for cysts or trophozoites of *G. lamblia*, or by examination of duodenal aspirates, which can yield more determinate results. The incidence of selective IgA deficiency in celiac disease has been demonstrated to be ten-fold higher than in the general population, given shared human leukocyte antigen (HLA) haplotypes [14]. Secretory IgA can bind to wheat gluten and gliadin, and the absence of IgA may lead to abnormal processing of these antigens [13]. The symptoms of celiac disease are similar in patients with or without IgA deficiency, the only differentiating feature is that immunohistochemical staining of small-intestinal biopsies reveals an absence of IgA-secreting plasma cells in IgA-deficient patients. Antigliadin IgA, antitissue transglutaminase IgA, and anti-endomysial IgA antibodies cannot be used as screening tests for this population; tissue transglutaminase IgG may be a better screening test [13]. Celiac disease associated with IgA deficiency is usually responsive to gluten withdrawal, failure response to a gluten-free diet should lead one to consider CVID [3, 13–15]. The histologically GI mucosa appearance is typically normal; however, especially in the duodenum, it can show nodular lymphoid hyperplasia (NLH) [13–15].

Multiple nodules are found in the lamina propria, superficial submucosa of the small intestine, or both, and occasionally can occur in the stomach, large intestine, or rectum. The lesions can be associated with mucosal flattening, causing malabsorption and even obstruction when large. In children, NLH tends to regresses spontaneously and is associated with mild symptoms; on the other hand, in adults, the prognosis is uncertain. Diagnosis is made by small-bowel endoscopy sometimes with the help of contrast barium or MRI studies [13–15]. Large amounts of IgM-bearing cells can be found in the immunohistochemical staining, possibly as compensation for the absent IgA. These patients may benefit from oral steroid therapy or, if *G. lamblia* or *H. pylori* infections occur, the eradication of that concomitant infections has been associated with a regression of NLH [13–15].

Persistent NLH has been associated with lymphomas, usually B-cell tumors and gastric carcinomas; so, in case of refractory NLH, a biopsy should be performed during endoscopy to rule out these diseases [15].

The diagnosis is based on at least one of the following:

- Increased susceptibility to infection
- Autoimmune manifestations
- Affected family member

AND diagnosis after fourth year of life

AND undetectable serum IgA (when measured with nephelometry less than 0.07 g/L) but normal serum IgG and IgM (measured at least twice)

AND secondary causes of hypogammaglobulinemia have been excluded

AND normal IgG antibody response to all vaccinations

AND exclusion of T-cell defect [7]

The threshold of 4 years of age is used to avoid premature diagnosis of IgA deficiency, which may be transient in younger children due to delayed ontogeny of IgA system after birth [11].

In IgA deficiency, the mainstay of the therapeutic approach is the treatment of associated diseases. If the patient experiences recurrent infections, daily prophylactic antibiotics on a continuous or seasonal intermittent basis may be beneficial [11]. IL-21 or the combination of CD40 L/anti-CD40, IL-4, and IL-10 is potential target for new therapeutic modalities [10, 16].

X-Linked Agammaglobulinemia

XLA is a primary immunodeficiency that results from a maturation arrest of pre-B cells with subsequent B cell generation failure, absence of plasma cell in lymphoid tissues, the virtual absence of all classes of Ig, small tonsils, and lymph nodes. The incidence is approximately 1 in 100,000 live births. The gene involved in XLA encodes for Bruton tyrosine kinase (BTK), and it is located on X-chromosome (Xq21.3-Xq22). It is an X recessive disease, so classically, it presents in male individuals, female carriers can be detected, and prenatal diagnosis of affected or unaffected male fetuses can be accomplished. There are about 554 different mutations of BKT gene. Similar phenotypes can result also from other mutations: autosomal-recessive agammaglobulinemia has been associated with mutations in genes that encode for μ heavy chain, λ5, Igα, Igβ, BLNK, PIK3R1, LRRC8, and TCF3. Autosomal-dominant mutations have been reported too [17, 18].

BTK is an intracellular tyrosine kinase protein expressed in most of hematopoietic cells: high levels are registered in all B-cell lines, no expression is reported in T cell precursors and natural killer (NK) cells. The abnormal BTK induces a maturational arrest of B lymphocytes at the pre-B cell stage, resulting in a normal number of pre-B cells in the bone marrow, but no mature B cells in the periphery. Peripheral $CD19^+$, $CD20^+$, and $CD23^+$ B cells are usually less than 0.1%.

IgG, IgA, and IgM levels are low or absent. There is no production of isohemagglutinins or antibody after vaccinations. The majority of males with XLA are asymptomatic during the first 4–6 months of life thanks to mother's transmitted antibodies; then, they present severe and repeated infections caused, mainly, by extracellular pyogenic and encapsulated organisms, often Gram positive (such as *S. pneumoniae, S. aureus, Neisseria, Haemophilus*, or *Mycoplasma*) [19].

The infections may interest respiratory, GI, and genitourinary tracts. Systemic infections (septicemia or meningitis) are less common but frequent, as well as osteomyelitis, septic arthritis, cellulitis, or skin abscesses. In such cases, we can observe chronic fungal infection or *Pneumocystis jirovecii* pneumonia. Viral infections are, usually, self-limiting except for hepatitis or enteroviruses despite a good T-cell response. GI manifestations are reported in 35% of individuals and can be so severe that parenteral nutrition and immunomodulatory therapy may be required [19].

The most common GI manifestations are intermittent diarrhea (38%), chronic diarrhea (26%), abdominal pain, gastroesophageal, reflux disease, and gastroenteritis [20]. Chronic diarrhea is often accompanied by secondary malabsorptive syndrome associated with a protein-losing enteropathy [21]. The main pathogens involved in these infectious diarrheas are *G. lamblia, Salmonella* spp., *Campylobacter jejuni,* and *Cryptosporidium parvum*; enteroviral infections (such as *Coxsackievirus* and *Echovirus*) are also common and can lead to severe neurologic defects. Sometimes chronic diarrhea is related to small bowel bacterial overgrowth. GI infection treatments must be based on adapted culture methods and adequate prolonged therapy. Cases of malabsorption and bacteremia due to infection of *Helicobacter pylori* and *Campylobacter jejuni* resistant to many antibiotics have been described [22].

It is important to highlight that the most common cause of death is chronic enteroviral infection [23, 24]. Inflammatory bowel diseases (IBDs) are common findings in XLA patients: the prevalence is 8 times higher compared to general population [20]. Some patients with XLA present small-bowel strictures and transmural intestinal fissures similar to Crohn's disease, without granulomas or plasma cells. XLA patients generally do not develop NLH. XLA occasionally manifests with different clinical spectrums such as autoimmune diseases or cancer (gastric adenocarcinoma and colorectal cancer). In particular, chronic atrophic gastritis with pernicious anemia is also a common finding that predisposes to gastric adenocarcinoma [23].

Diagnosis is based on clinical features and typical laboratory results: fewer than 2% circulating B cells (CD19 and CD20), preferably in two separate determinations and a normal number of T cells (CD3, CD4 and CD8).

AND serum IgG levels below:

−200 mg/dl in infants aged <12 months
−500 mg/dl in children aged >12 months

OR normal IgG levels with IgA and IgM below 2SD
AND onset of recurrent infections before 5 years of age [7].
It could be confirmed by molecular analysis [19].

Early diagnosis of XLA with immediate initiation of therapy is crucial for ensuring good outcomes for the affected patients. A delayed diagnosis could lead to long-lasting sequelae such as bronchiectasis, hearing loss, or liver cirrhosis due to chronic hepatitis. XLA treatment consists of replacement IgG therapy, either intravenous (intravenous gammaglobulin [IVGG]) or subcutaneous. Early IVGG replacement therapy decreases the rates of admission and morbidity for chronic complications, such as bronchiectasis

and chronic lung disease, and prevents fatal complications like meningoencephalitis. In addition, IVGG may represent also a potentially beneficial treatment for Crohn's disease in these individuals [19]. Appropriate IVGG should be started at 6–8 weeks of age, because around 25% of the XLA patients show clinical symptoms before 4 months of age. Antibiotics treatment for documented or suspected infections is necessary, because commercial preparations of IgG could not have adequate titers against uncommon organisms.

Hyper-IgM Syndrome

Hyper-IgM syndromes are a group of rare inherited immunodeficiencies characterized by impairment in B cell class switch recombination (CSR) and somatic hypermutation (SHM) resulting from defects in the CD40 ligand/CD40 signaling pathway. They are characterized by high levels of IgM associated with low or absent levels of IgG, IgA, and IgE [25, 26]. Several variants of hyper-IgM syndrome have been described and result in different phenotypes:

1. *X-linked hyper-IgM syndrome*

It is the most common form accounting for almost 65–70% of all cases. This condition could derive from two different gene mutations.

The first gene encodes for the CD40 ligand (CD40L) expressed on T-cells, and it is defined as hyper-IgM syndrome type 1(HIGM1). CD40L is a member of tumor necrosis factor (TNF), and mutations are typically reported on the extracellular domain that interacts with CD40. CD40 is expressed on APCs (B cells, monocytes, macrophages, and dendritic cells), and mutations of CD40 are associated with autosomal-recessive hyper-IgM syndrome type 3 (HIGM3). Because of the lack of this ligand on T cells, there is no interaction with CD40 on B cells and impossibility to go through CSR or SHM. B cells cultured can produce not only IgM, but also IgG, IgA, and IgE: this confirms that the defect interests T cells. Mitogen proliferation may be normal, but NK cell and T cell cytotoxicities are frequently impaired. Antigen-specific responses may be decreased or absent. Males with this syndrome present small tonsils, absence of palpable lymph nodes. Neutropenia, thrombocytopenia, and anemia are common [25]. The second gene, located on X-chromosome, is NEMO; it encodes for a nuclear factor-kappa B (NF-kB) nuclear factor [25].

This factor acts with IKBA/IκBα, they are involved in the pathway activated by CD40L-CD40; autosomal mutations of IKBA/IκBα lead to a similar phenotype. Mutations of NEMO are described mainly in the C-terminal domain. In males, this form is clinically associated with anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID); in females, it causes incontinentia pigmenti [25].

2. *Autosomal recessive hyper-IgM syndrome*

This is less common compared to X-linked type. The most common forms are caused by defects in the CD40-activated RNA-editing enzymes AID and UNG, which are required for CSR and SHM. AICDA encodes for AID and it is located on chromosome 12, mutations of this protein lead to HIGM2; UNG gene is located on chromosome 12, and mutation of it causes HIGM5 [26]. During CSR and SHM, AID generates deoxyuracils in the switch regions of Ig heavy chain, then UNG removes deoxyuracils generating double-strand breaks.

In this case, B cells cultured are not able to produce all classes of Ig; this confirms that there is a real defect in the B cell; however, no alteration in the numbers of CD19+ B cells, CD27+ memory B cells, and T-cell immunity is found [25, 26].

3. *Autosomal dominant hyper-IgM syndrome*

Other genes can cause similar phenotype: heterozygous gain-of-function mutations in PI3K, a kinase activated by CD40-CD40L, belong to this group [25].

Distinctive clinical features for these patients allow presumptive recognition of mutation, this is important to choose the best therapy.

The range of clinical findings varies, even within the same family.

Over 50% of males with HIGM1 develop symptoms by the age of 1 year due to the presence of maternal antibodies, and more than 90% are symptomatic by the age of 4 years. The clinical presentation of the hyper-IgM syndrome is similar to XLA, with recurrent pyogenic infections such as otitis, sinusitis, pneumonia, or tonsillitis that start in the first 2 years of life in patients regardless of the type of mutation [25, 26].

In addition, patients that presented mutations on CD40-CD40L genes are also susceptible to a variety of opportunistic and intracellular pathogens such as mycobacterial species, fungi, and viruses; in about 40% of cases, these individuals present pneumonia by *Pneumocystis jirovecii*. Significant neurologic complications are seen in 10–15% of males with HIGM1. In at least 50%, a specific infectious agent cannot be isolated [25, 26].

In patients with mutations that do not involve CD40-CD40L genes, the risk of opportunistic infection is lower because of unaffected T-cell immunity.

GI symptoms are the second most common symptoms and include mostly chronic diarrhea and liver involvement, and it occurs in almost one-third of HIGM1 patients [27].

The main pathogens that cause diarrhea are *Cryptosporidium parvum* (the most common), *G. lamblia, Salmonella*, or *Entamoeba histolytica.*

GI complications can cause failure to thrive and weight loss.

Neutropenia often causes oral or rectal ulcers and gingivitis or mucosal abscess, and it is common in patients with mutations involving CD40 complex.

Liver disease, a serious complication of HIGM1, historically, was observed in more than 80% of affected males by age 20 years. Cholangiopathy with *Cryptosporidium* in the biliary tree, B and C hepatitis, and cytomegalovirus (CMV) infections with a possible evolution in cirrhosis or hepatocellular carcinoma are common findings [25]. Hepatic involvement is associated with high gamma-glutamyltransferase levels; this alteration can predict a possible development of sclerosing cholangitis with a risk of cholangiocarcinoma. Liver tumors account for approximately 25% of mortality in X-HIGM.

Screening for patients with sclerosing cholangitis by magnetic resonance cholangiopancreatography (MRCP) and serum cancer antigen 19–9 levels every 6 months have been suggested [27].

Gastrointestinal diseases are also presented less severely in AID-/UNG -deficient patients when compared to X-HIGM.

Tumors of the GI tract (carcinoid of the pancreas, glucagonoma) are common life-threatening complications in adolescents and young adults with HIGM1 [26–29]. Affected males also have an increased risk for lymphoma, particularly Hodgkin's disease associated with Epstein–Barr virus infection. In individuals with AID or UNG mutations, enlarged lymph nodes and hepatosplenomegaly, especially during early childhood, can be apprised; they result probably from overactivated germinal centers, and these conditions have been associated with a higher risk of lymphoma [27].

In addition, spontaneous rib fractures are frequent among HIGM1 patients, probably due to a T-cell dysfunction osteopenia.

Autosomal-recessive forms have usually a later onset and are often associated with a higher rate of autoimmune disorders (such as diabetes mellitus, autoimmune hepatitis, autoimmune thrombocytopenia, and Crohn's disease). Neutropenia is less common [27, 28].

The reported median survival of males with HIGM1 who did not undergo a successful allogeneic hemopoietic stem cell transplantation (HSCT) is less than 25 years, and liver disease is the main predictor of overall outcome and mortality [28].

P. jirovecii pneumonia in infancy, liver disease, and carcinomas of the liver and GI tract in adolescence or young adulthood are the major causes of death [29].

Laboratory evaluation is fundamental: these patients have low or absent levels of IgG, IgE, and IgA associated with normal or high levels of IgM (very high levels of IgM are typical of the autosomal-recessive form) and IgD. B and T lymphocyte levels are usually normal. The diagnosis of HIGM1 is based on a combination of clinical findings, family history, absent or decreased expression of the CD40 ligand (CD40L) protein on flow cytometry following in vitro stimulation of white cells, and molecular genetic testing of CD40LG (previously known as TNFSF5 or CD154).

The only curative treatment currently available is allogeneic hematopoietic cell transplantation, ideally performed before the onset of life-threatening complications and organ damage. No difference in survival rate between patients that received transplant and patients that were treated with IVGG has been apprised in patients with X-linked mutation [26]. Gene-editing may be a future approach [29].

Other effective therapies are the monthly replacement of Ig and antibiotics for specific infectious complications. To reduce the risk of *Cryptosporidium* infection, it is recommended that patients boil or filter water. In patients with neutropenia, it is possible to use granulocyte colony–stimulating factor (G-CSF) [26–29].

Common Variable Immunodeficiency

Common Variable Immunodeficiency (CVID) with an estimated prevalence of 1/100,000 to 1/50,000 is the most common symptomatic primary antibody-deficient syndrome.

The diagnosis is based on at least one of the following:

- Increased susceptibility to infection
- Autoimmune manifestations
- Granulomatous disease
- Unexplained polyclonal lymphoproliferation
- Affected family member with antibody deficiency

AND marked decrease of IgG and marked decrease of IgA with or without low IgM levels (measured at least twice; <2SD of the normal levels for their age).

AND at least one of the following:

- Poor antibody response to vaccines (and/or absent isohemagglutinins); i.e., absence of protective levels despite vaccination where defined
- Low switched memory B cells (<70% of age-related normal value)

AND secondary causes of hypogammaglobulinemia have been excluded [7].

The genetic study is not mandatory but should be encouraged [30].

Most patients are diagnosed with CVID between the ages of 20 and 40 years; however, the diagnosis commonly is delayed by 6–8 years, even after the onset of characteristic symptoms [30].

The genetic basis of CVID is unknown in most cases (85%) and the impairment of B cells can occur at five different stages: B-cells production; early peripheral B-cells maturation or survival; B-cells activation and proliferation; germinal center; postgerminal center.

The defect can be either of B cell either of other cells committed in B-Cell activation. Not only B cells and plasma cells are typically low, also CD4, NK, and other cells levels can be reduced.

In only 5–15% autosomal-dominant/autosomal-recessive inheritance has been described, and the genes implied are inducible T cell costimulator (ICOS), tumor necrosis factor receptor superfamily member 13B (TNFRSF13B, also known as TACI), tumor necrosis factor receptor superfamily member 13C (TNFRSF13C, also known as BAFFR), CD19, CD20, CD21, CLTA4 [31].

GI symptoms are the second most common symptoms after sinus-pulmonary infections in CVID patients, as up to 50% of patients have chronic diarrhea with malabsorption; malabsorption is one of the main responsible reasons for nonmalignant mortality [31]. GI problems can be either infective or inflammatory.

Recurrent intestinal infections are mainly caused by *G. lamblia, C. jejuni,* or *Salmonella*, pathogens frequently associated with profound B cell immunodeficiencies [32].

Noninfective diarrhea and malabsorption have also been ascribed to the onset of IBD or intestinal villous atrophy mimicking celiac disease, a condition observed in 31% of CVID patients with GI symptoms or anemia [15]. Sometimes celiac disease is present too and the differential diagnosis is difficult; antibodies (antitissue transglutaminase, antigliadin, and anti endomysial) are not useful; on the other hand, the genetic study of HLA and the absence of plasma cells in the mucosa can be helpful to differentiate villous atrophy and celiac disease; the response to gluten-free diets is not always satisfactory [15].

CVID mucosa alterations are characterized by the lack of eosinophils, diffuse superficial lesion with apoptotic bodies, and high levels of lymphocytes in crypts. These features are histologically indistinguishable from acute graft-versus-host disease (GVHD) [32].

Liver involvement has been described in 10% of CVID patients; autoimmune reaction, infection, and malignancy are the most common conditions. A biopsy may be necessary to diagnose liver autoimmune disease, since often, the autoantibodies are low [33].

Atypical sarcoid-like lesions are described in 8–22% of patients with CVID; they typically can be appraised in lungs, lymph nodes even in the spleen, and in the GI tract [31].

Atrophic gastritis, which resembles autoimmune gastritis developing into pernicious anemia, may occur in the absence of demonstrable antiparietal cell antibodies in CVID patients.

Treatment of pernicious anemia in CVID consists of monthly replacement of vitamin B12 and careful monitoring of the gastric mucosa for changes associated with malignancy. Gastric adenocarcinoma, in the setting of atrophic gastritis or *H. pylori* infection or de novo, has an increased frequency in CVID patients [31, 32].

Several studies have been done to evaluate a possible correlation between alteration of microbiota and phenotype severity among CVID; a positive association between severe phenotype and higher microbiota dysbiosis has been apprised [34].

Patients are treated with monthly infusions of IgG; this treatment alone may not be effective in treating other manifestations of this disorder. Although the inflammatory process responds to steroid therapy, prolonged therapy with steroids is not advisable for CVID patients. Other immune modulators such as 6-mercaptopurine (6-MP) or azathioprine (AZA) can be used in addition to Ig replacement therapy. Careful use of budesonide may be required if unresponsive to 6-MP or AZA until the symptoms are controlled (e.g., reversal of weight loss and dehydration). In severe cases of malabsorption, when a significant loss of essential nutrients (e.g., calcium, zinc, and vitamins A, E, and D) leads to bone loss and neurologic deficits, limited use of total parenteral nutrition may be required [31, 32].

Combined T and B Cell Immunodeficiency

Severe Combined Immunodeficiency

Severe Combined Immunodeficiency (SCID) includes a group of disorders characterized by impaired development of the cellular and humoral immune functions. The overall incidence of all types of SCID is approximately 1 in 58,000 births [35]. This condition is uniformly fatal in the first 2 years of life unless immune reconstitution can be accomplished. The common characteristic of all types of SCID is a severe impairment of T cell development, with a virtual lack of circulating autologous T lymphocytes and the absence of functional T-cell responses. SCID can be divided into two main classes: with (B + SCID) and without (B − SCID) B lymphocytes and a further subdivision based on the presence of NK in NK+/NK−. However, a new classification is needed based on the novel discoveries: SCID with a normal number of T cells (T+) but a deficit in the T-cell intracellular signaling, resulting in a functional impairment, has been described [36, 37].

SCID results from mutations in known genes that encode components of the immune system crucial for lymphoid cell development (Table 38.5).

The phenotype can vary from severe combined immunodeficiencies (SCID) to some mild form defined as combined immunodeficiencies (CIDs).

Moreover, when the mutated gene is implied in the V(D)J recombination, these mutations result in radio-sensibility (RS) and are defined as RS-SCID [36].

Affected individuals appear to be normal infants at birth but begin to suffer from excessive infections and failure to thrive coincident with the waning of transplacentally acquired maternal IgG between 2 and 4 months of age and, without any treatment, death occurs typically by the first 2 years of life [37].

Most males with typical X-SCID come to medical attention between ages 3 and 6 months.

During the first year of life, nearly all untreated males with X-SCID present failure to thrive, oral/diaper candidiasis, absent tonsils, and lymph nodes, recurrent and persistent infections, despite conventional treatments, especially from opportunistic organisms such as *P. jirovecii*. Additional features include immune dysregulation and autoimmunity-associated with rashes, GI malabsorption, and short stature: 10–15% of patients have a "delayed" clinical onset by age 6–24 months and a smaller percentage of patients have "later"'onset, diagnosed from ages 4 years to adulthood, showing less severe infections, and gradual immunologic deterioration [36].

All patients with SCID have very small thymuses. The spleen appears depleted of lymphocytes. Lymph nodes, tonsils, adenoids, and Peyer patches are absent or extremely underdeveloped. GI disorders often occur in SCID patients. Oral, esophageal, and perianal candidiasis is common and can affect oral intake [3, 13, 15].

Intractable diarrhea may begin slowly and progress to massive, watery, bloody, and mucopurulent. The stool should be sent to a laboratory to check for viral and opportunistic infections, especially rotavirus. Chronic infection with rotavirus has been reported in patients who have received the live vaccination. CMV and adenovirus infections also have been identified in GI biopsy specimens. GI biopsy specimens show hypocellular lamina propria, without plasma cells or lymphocytes. Villous atrophy may occur in some infants probably secondary to the damage of the intestine after viral or bacterial infections [13, 15]. A hereditary multiple intestinal atresia is described as an unusual and rare form of recurrent intestinal atresia, which can be associated with SCID [34, 35]. Graft versus host disease (GVHD) and a GVHD-like process affecting colon and small intestine can occur in patients with SCID who received blood transfusions or allogeneic bone marrow transplantation. Early recognition and diagnosis are crucial for these patients. The diagnosis is based on at least one of the following:

- Invasive bacterial, viral, or fungal/opportunistic infection
- Persistent diarrhea and failure to thrive
- Affected family member

AND manifestation in the first year of life
AND HIV excluded
AND 2 of 4 T cell criteria fulfilled:

- Low or absent CD3 or CD4 or CD8 T cells
- Reduced naive CD4 and/or CD8 T cells
- Elevated g/d T cells
- Reduced or absent proliferation to mitogen or TCR stimulation [7]

The number of B and NK cells varies according to the underlying genetic defect. In rare cases, maternal engraftment of T cells may cause near-normal T cell counts. Serum levels of Ig are usually very low, and specific antibody responses are impaired.

Diagnosis of infants is often delayed by several months as a result of the protection of maternal antibodies; however, after the introduction of the screening, an advanced diagnosis has been done. Since 2018, in all states of USA, quantitative polymerase chain reaction (PCR) measurement of T-cell receptor excision circles (TRECs) has been introduced. TRECs are indicators of T-naive and are not produced by T mature cell; a control (b-actin) is measured to differentiate samples with true TREC levels from samples with poor quantity [35].

If the screening indicates a possible SCID, the next exam is flow cytometry that confirms the diagnosis. When there is a prior SCID-affected child with a known specific molecular defect, prenatal diagnosis can be made through genetic testing. After diagnosis, treatment relies on the prevention of principal complications until allogeneic HSCT, which is the definitive treatment [38, 39]. Replacement Ig therapy is given, as well as prophylaxis for *P. jirovecii* pneumonia. Live vaccinations should not be given to the patient or the patient's caregivers, because they can cause life-threatening infections. Blood products are irradiated before administration to prevent GVHD [36].

The genetic diagnosis is not mandatory before proceeding with HSC; however, for some specific mutations (SCID-X1 and adenosine deaminase (ADA-SCID)), gene therapy and enzyme replacement therapy (ADA-SCID) are possible options, so genetic diagnosis should be promoted in case of the suspect of these mutations [35]. Without treatment, death usually occurs in the first 2 years; with HCT cell transplantation, the 2-year survival rate was approximately 95% in

infants without previous infections, 81% in those with previous infections.

A better outcome is associated with an early HSC; in patients receiving HSC under 3.5 months of age and prior to infection, the 5-year survival was 80–95% [39, 40].

Disorder of Phagocytes Function

Chronic Granulomatous Disease (CGD)

CGD is an uncommon primary immunodeficiency (affecting 1 in 250,000 live births) caused by an inability of the phagocytes to produce adequate reactive oxygen metabolites to kill ingested microorganisms and leads to recurrent or persistent intracellular bacterial and fungal infections and to granuloma formation. This impairment in killing is caused by any of the several defects that may occur in one of the five genes that encode for the five subunits of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex also defined as NOX2, which generates the microbicidal respiratory burst [41].

The most common form is X-linked, involving the gp91phox subunit, that, with the autosomal-recessive form, involving the NCF1 subunit, accounts for 90% of CGD. Autosomal-recessive mutations of the other three genes encoding for p22phox, p67phox, and p40phox are responsible for the 10% of CGD [41].

Patients with CGD are susceptible to severe and recurrent infections from catalase-positive organisms (e.g., *Staphylococcus aureus, Burkholderia cepacia, Serratia marcescens, Aspergillus species, Chromobacterium violaceum,* and *Nocardia* species) at epithelial surfaces (e.g., skin, gut, lungs) as well as in organs with a large number of phagocytes such as the liver.

The disease becomes apparent during the first 2 years of life in most patients. Besides infections, these individuals suffer from inflammatory manifestations that are twice more common in X-linked GCS [41].

The classic inflammatory finding is the granuloma; granulomas are noncaseating, matched with fibrotic tissue; they typically affect hollow viscera, are not related to infection, and tend to respond to steroid therapy [41].

GI clinical manifestations are reported in almost 50% of patients with CGD and can sometimes be the first symptom. Up to one-third reports diffuse or lower abdominal pain; the main manifestations reported are chronic diarrhea, malabsorption with steatorrhea and vitamin B12 malabsorption, perianal abscesses, fistulae, GI tract infections, liver abscesses, hepatosplenomegaly, oral ulceration, noninfective esophageal ulcers, esophageal dysmotility, and characteristic obstructive lesions associated with granulomatous infiltration [42].

CGD subjects with inflammatory bowel disease (IBD) often present with signs and symptoms similar to those seen in Crohn's disease (CD) and ulcerative colitis (UC). However, in contrast to CD and UC, CGD-IBD may have distinctive histopathologic findings: in acute and chronic colitis, the main findings are crypt abscess and lymphoplasmacytic with more eosinophils, fewer neutrophils, and numerous lipid-laden macrophages located in the lower third of the mucosa near crypt bases. Macroscopically, in CGD-IBD, the inflammation involves the rectum, is transmural, and is not associated with dysplasia. CD-associated antimicrobial antibodies can be indiscriminately high in patients with CGD with or without GI problems and are useless to differentiate CD from CGD-IBD. It is critically important for the clinician to consider the possibility of CGD in patients with a "Crohn-like" disease in whom a history of recurrent infections and abscesses is noted [42].

Diagnosis of CGD is made by demonstrating a lack of oxidative burst by the nitroblue–tetrazolium test or a flow cytometric test using dihydrorhodamine (DHR) dye and confirmed by Western blot and molecular analysis. Individuals with <10% DHR oxidase activity have a high risk of infection and need therapy. In addition, some authors suggest promoting the same management in individuals with 10–20% DHR activity due to a still high risk of infection [43].

The diagnosis of CGD needs also at least one of the following features:

- Deep-seated infection due to bacteria and/or fungi (abscesses, osteomyelitis, lymphadenitis)
- Recurrent pneumonia
- Lymphadenopathy and/or hepatomegaly and/or splenomegaly
- Obstructing/diffuse granulomata (gastrointestinal or urogenital tract)
- Chronic inflammatory manifestations (colitis, liver abscess, and fistula formation) [7]

Management of CGD includes antibiotic prophylaxis with trimethoprim-sulfamethoxazole and itraconazole to reduce the incidence of bacterial and fungal infections, although breakthrough infections do occur. Prophylactic administration of subcutaneous interferon-γ also has been used [44].

CGD-IBD typically responds rapidly to treatment with steroids followed by the addition of a salicylic acid derivative and/or a purine antimetabolite. Additional therapy used form CGD-IBD are hydroxychloroquine, anakirna, and TNFα inhibitors; TNFα inhibitors can lead to severe life-threatening infections, so they must be used with caution. In some severe form, bowel surgery may be needed [42, 43].

Hematopoietic stem cell transplantation from an HLA-identical donor can be curative for CGD and gene therapy may be an option in the future [44]. More than 50% remains alive ≥25 years after diagnosis [41].

Immune Dysregulation Diseases

IPEX and IPEX-Like Disorders

IPEX and IPEX-like disorders are a group of disorders characterized by immune dysregulation and autoimmunity that result from defects in T regulatory cells, development, and function. The main disease of this group is "immune dysregulation, polyendocrinophaty, enteropathy, X-linked (IPEX)," resulting from mutations affecting FOXP3.

The diagnosis of IPEX is based on at least one of the following:

- Severe and protracted enteropathy with villous atrophy in a male infant
- Severe, often multiple endocrinopathies

AND exclusion of hypogammaglobulinemia
AND at least one of the following:

- Low or absent Foxp3 expression by CD4 + CD25+ on flow analysis
- No overt T cell defect (proliferations are normal)
- Elevated IgA and IgE levels
- Normal CD25 expression [7]

Several other gene defects that affect T-regulatory cell function also give rise to an IPEX-like phenotype. Some are autosomal recessive and involve the following genes: LRBA, CD25, ITCH, CTLA4, IL2RA, STAT5B, DOCK8, MALT-1; autosomal-dominant mutations involve STAT1 gene and STAT3 gene; these last two are the most common and account for almost 38%. Table 38.6) [45–49].

In addition, two other genes have been associated with IPEX-like syndrome (TTC37, TTC7A); homozygous mutation of TTC37 results in trichohepatoenteric syndrome characterized by growth restriction, diarrhea, trichorrhexis nodosa-like hair morphology, hepatopathy, facial dysmorphism, and immunodeficiency. TTC7A homozygous mutation results in IPEX-like disorder and multiple intestinal atresia [47].

The diagnosis of IPEX-like is based on at least one of the following:

- Severe and protracted enteropathy with villous atrophy in a male infant
- Severe, often multiple endocrinopathies

Table 38.6 Clinical and laboratory features of IPEX and IPEX-like disorders

	IPEX	CD25	STAT5b	STAT-1	ITCH	DOCK8	MALT-1
Associated features	None	None	Growth failure	Vascular anomalies	Dysmorphic growth failure	Growth failure	Growth failure, facial dysmorphy
Autoimmunity							
Eczema	+++	+++	++	++	++	+++	+
Enteropathy	+++	+++	++	++	++	++	++
Endocrinopathy	+++	++	+	++	++	++	+
Allergic disease	+++	+	+	++	++	+	+
Lung disease	+	++	+++	+	+++	Unknown	++
Infections							
Yeast	–	++	–	+++	–	++	+
Herpes virus	–	+++(EBV/CMV)	++(VZV)	++	–	++	+
Bacterial	+/–	++	++	++	+	+	+
Laboratory findings							
Cytopenias	++	++	++	–		+	–
Serum immunoglobulins	↑	Normal or ↑	Normal or ↑	Normal or ↑/↓	↑	Normal or ↑/↓	Normal or ↑/↓
Serum IgE	↑	Normal or ↑	Normal or ↑	Normal or mildly ↑	↑	↑	↑
CD25 expression	Normal	↓	Normal or ↓	Normal	Unknown	Normal or ↓	Normal
CD4 + CD45RO	↑	↑	↑	Normal or ↑	Unknown	Normal or ↑	↑
FOXP3 expression	Normal or ↓	Normal or ↓	Normal or ↓	Normal	Unknown	Normal	↓
IGF-1, IGFBP-3	Normal	Normal	↓	Normal	Unknown	Unknown	Unknown
Prolactin	Normal	Normal	↑	Normal	Unknown	Unknown	Unknown

CMV cytomegalovirus, *EBV* Epstein-Barr virus, *VZV* varicella zoster virus, *IGF* insulin-like growth factor–binding protein, *IGFBP* insulin-like growth factor–binding protein [45–49]

AND exclusion of hypogammaglobulinemia
AND at least one of the following:

- Normal Foxp3 expression by CD4 + CD25+ on flow analysis
- No overt T cell defect (proliferations are normal)
- Elevated IgA and IgE levels [7]

IPEX typically presents during the first 2–3 months of life, IPEX-like disorders around 17 months of life; in some cases, the symptoms can start at birth or even in delayed childhood or adolescence. A correlation between genotype and phenotype has not been established; however, IPEX nonsense and frameshift mutations have been associated with a more severe phenotype [50].

The initial triad of symptoms is classically characterized by secretory diarrhea (at times mucoid or bloody) unresponsive to fasting, eczema, and diabetes mellitus. Clinical presentation can be similar to severe food allergy, although these patients are generally unresponsive to the elemental formula. Three distinct histological patterns have been described: they are not mutually exclusive: graft-vs-host disease-like pattern, celiac disease-like pattern, and enteropathy with intestinal goblet cell autoantibodies pattern [50]. The main histologic features include total or partial villous atrophy, lymphocytic, eosinophil, plasma cell infiltration in the lamina propria, and destruction of mucosal, submucosal, and muscular layer sparing.

Antienterocyte antibodies are usually present; however, they are considered accessory findings rather than pathogenetic factors. The GI problems lead to severe alterations of the nutritional status and glucose metabolism. Skin involvement can range from mild to severe manifestations, it usually does not respond to treatment and there is a high risk of bacterial superinfection. Other autoimmune manifestations, including hyperthyroidism, hypothyroidism, autoimmune hemolytic anemia, thrombocytopenia, and neutropenia, have been reported [51].

B-cell subsets and CD3+ T cells, including CD4+ and CD8+ subsets, are present in normal numbers and proliferate to mitogens and antigens, although affected patients typically have decreased number of regulatory T-cells and are unable to suppress T-cells proliferation.

Serum IgG, IgA, and IgM levels are usually normal but can be slightly reduced from enteric protein loss. IgE levels usually are increased, and eosinophilia is frequently present. The diagnosis is made by showing decreased FOXP3 protein expression and a reduction in the number of regulatory T cells. The diagnosis also can be confirmed by mutation testing of the FOXP3 gene [47]. A mutation on FOXP3 is appraised in 50% of cases; in the other 50%, the mutation involves one of the causative IPEX-like disorder genes [47].

Autoantibodies to harmonin and villin are considered specific diagnostic markers of IPEX and could be of help to differentiate IPEX, including atypical cases, from other early disorders associated with severe enteropathy. In addition, they become low in patients well controlled either with immunosuppression (IS) or HSCT [51].

Patients are often severely ill by the time a diagnosis of IPEX is made. In the absence of aggressive therapy, children usually die before 2–3 years of age as a result of malnutrition, electrolyte imbalance, or infection [51]. No differences between IPEX and IPEX-like in long-term survival have been reported [51].

Symptomatic treatment includes bowel rest with total parental nutrition and, if necessary, insulin injections and red blood cell and platelet transfusions. The only curative treatment available is HSCT with 15 years survival rates of 73.2%. Immunosuppression (IS) has been used as an alternative to HSCT; the most effective immunosuppressant is rapamycin followed by cyclosporine, tacrolimus, sirolimus, and steroids. IS therapy is associated with a higher 15 years survival rate (86.6%); however, IS is not curative and chronic therapy may be toxic and may facilitate opportunistic infections [50, 51]. Gene therapy may be an option in the future.

Interleukin-10 and Interleukin-10-Receptor Defects

IL-10 pathway plays a critical role in the control of inflammation by limiting the secretion of pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, and IL-12. Homozygous loss-of-function mutations of the genes that encode for either IL-10 or IL-10 receptor (IL-10R) have been associated with very early-onset IBD (VEO-IBD). IL-10R is a tetrameric receptor composed of two alpha-unit (IL-10R1) encoded by IL-10RA and two beta-unit (IL-10R2) encoded by IL-10RB; IL10RA is specific for IL-10, ILRB can bind also IL-22, IL-26, IL-28, and IL-29 [52].

Mutations in these genes have been apprised in 4–25% of VEOIBD; a Korean study reports a mutation in ILRA in 50% of IBD diagnosed within the first year of age [53, 54].

These patients present within the first year of life with enterocolitis and perianal disease, as well as the formation of multiple abscesses and entero-cutaneous fistula, requiring several surgical interventions.

The microscopic appearance of the mucosa is characterized by the presence of ulcer lesions with eosinophils, inflammatory infiltrates in the epithelium, cryptic abscesses extending to the muscularis mucosa, and drop-out necrosis [54].

Patients also suffer from chronic folliculitis and recurrent respiratory infections.

Skin and respiratory infections are more common among IL-10RB mutation, since it binds IL-22 that is involved in skin and lung epithelial immunity.

Hearing loss has been reported too and might be caused by the multiple high respiratory tract infection. In addition, EBV-associated lymphomas have been described among individuals with IL10RB mutation probably because IL10RB is implied in the γ-interferon pathway [53–55].

Although rare, pediatric patients with IBD and perianal disease should be screened for IL-10 and IL-10R deficiency. Defects in the IL-10R may be evaluated by functional assays.

Peripheral blood mononuclear cells from healthy individuals show strong phosphorylation of the signal transducer and activator of transcription-3 (STAT3) upon stimulation with IL-10, whereas peripheral blood mononuclear cells from IL-10R deficient patients do not. Functional abnormalities can be confirmed by sequencing IL-10RA and IL-10RB [56].

In addition, decreased CD41/CD81 T-cell ratio; high, low, or normal NK; B; T cell; increased or decreased serum immunoglobulin levels that may require immunoglobulin substitution have been reported. No genotype–phenotype correlation has been described.

The management of these patients includes various anti-inflammatory drugs, such as steroids, methotrexate, thalidomide, anti-TNFα monoclonal antibodies, and even fecal microbiota transplantation. However, in many cases, no remission or long-term improvement is achieved.

Surgery may be needed in case of high inflammation.

Both IL-10 and IL-10 receptor deficiency can be successfully treated by HSCT [57].

Immunodeficiency-Associated with Other Defects

Wiskott Aldrich Syndrome (WAS)

WAS is a rare X-linked primary immunodeficiency characterized by microthrombocytopenia, eczema, recurrent infections, and an increased incidence of autoimmunity and malignancies. In the USA, the incidence has been estimated at 1:250,000 male births.

The disease is caused by mutations in the WASp gene, which is involved in actin polymerization cytoskeletal remodeling during the hematopoietic cell differentiation and development [58]. A 5-point severity score has been introduced to differentiate patients with milder presentation (score <2) from those with a severe or well-defined classic type (>3–5) [58, 59].

The most severe form of WAS is associated with mutations that lead to absent or truncated protein, and individuals with this class of mutations develop the classic WAS.

Mutations that lead to full-length, but reduced quantity of WASp present thrombocytopenia with minimal, if any, immunodeficiency.

Missense mutations that do not affect WASp expression and lead only to intermittent thrombocytopenia.

The fourth class of mutations is caused by a missense mutation in the WASp Cdc42-binding site.

Of note, platelets have reduced size in the first three classes of mutations described as the pathognomonic sign of the diseases and no tendency toward neutropenia. Individuals with the fourth class of mutations demonstrate no thrombocytopenia and normal-sized platelets, but they typically develop neutropenia (and often a complete loss of this cell line) with infections [59].

Thrombocytopenia results form more than one pathogenic pathway: accelerate peripheral destruction, antiplatelets antibodies, and impaired functionality. Interestingly, megakaryocytes are normal or increased in numbers in the bone marrow and normal platelet production is apprised in vitro.

WAS can also be associated with other immune defects, including abnormalities of humoral and cell-mediated immunity. Patients with WAS usually are male infants presenting in the first few months of life with the above clinical picture accompanied by bleeding and bloody diarrhea. Although GI complications are not prominent, malabsorption and nonspecific colitis may be encountered, and higher rates compared to the general population of IBD and IBD-like disease have been reported [3, 13, 15]. Serious infections also occur. Encapsulated organisms are frequent pathogens that may cause life-threatening complications, including pneumonia, meningitis, and sepsis. Severe presentations of viral infections from VZV, HSV, EBV, CMV, and HPV have been reported [58, 59].

Opportunistic infections can also present under the form of *Pneumocystis jirovecii*.

Atopic symptoms are frequently present, and eczema develops in more than 2/3 of these patients.

Eczema may improve as the patient gets older, although serious complications such as secondary infection (e.g., cellulitis, abscess) or erythroderma can occur. Autoimmune manifestations are really common (70%): cytopenias, arthritis, vasculitis, IgA nephropathy, and Henoch-Schönlein purpura have been reported [59].

A higher incidence of malignancy has been described, predominantly non-Hodgkin type and often EBV-induced [59].

Laboratory evaluation reveals normal or slightly low IgG levels, high IgA and IgE levels, and low levels of IgM that are secondary to accelerated synthesis and catabolism.

Antibody responses to polysaccharide antigens are decreased. Platelet numbers are reduced and small in size. Patients have low B cell levels during infancy that normalize in adulthood, while their T cells show a progressive decrease

in number and function. Diminished lymphocyte proliferation in response to mitogens occurs in approximately 50% of patients. The number and phagocytic activity of neutrophils are normal, although chemotactic responses are defective.

The diagnosis is based on at least one of the following:

- Eczema
- Recurrent bacterial or viral infections
- Autoimmune diseases (incl. vasculitis)
- Malignancy
- Reduced WASP expression in a fresh blood sample
- Abnormal antibody response to polysaccharide antigens and/or low isohemagglutinins
- Positive maternal family history of XLT/WAS AND male patient with thrombocytopenia (less than 100,000 platelets/mm^3) (measured at least twice)

AND small platelets (platelet volume <7.5 fl) [7]

Screening for WASp mutations is performed by flow cytometry; however, this does not identify carriers of, or those patients with, X-linked thrombocytopenia. Sequence analysis of the WAS gene is essential to confirm the diagnosis [59]. Curative therapy for WAS is hematopoietic cell transplantation and lentiviral vector–mediated HSPC gene therapy [59, 60]. Conventional management includes antibiotic prophylaxis as well as platelet transfusions in severe situation; prophylactic transfusions to maintain normal platelet counts are discouraged, since it can result in the development of anti-HLA antibodies and should be used only in a life-threatening situation [59, 60].

Hermansky–Pudlak Syndrome (HPS)

HPS is a rare autosomal-recessive disease that displays genetic heterogeneity; there are ten known subtypes (HPS1-10) [61]. All subtypes are characterized by tyrosinase-positive oculo-cutaneous albinism and bleeding diathesis that results from a platelet storage pool deficiency.

HPS1-2-4 may present also with pulmonary fibrosis and HPS1-4-6 with granulomatous colitis. HPS gene is ubiquitously expressed and encodes for a molecule that is critical in trafficking to lysosome-like organelles like melanosomes and platelet dense granules [61].

Systemic complications are associated with the accumulation of ceroid lipofuscin.

The bleeding diathesis can result in easy bruising, frequent epistaxis, gingival bleeding, postpartum hemorrhage, colonic bleeding, and prolonged bleeding with menses or after tooth extraction, circumcision, and other surgical procedures. Pulmonary fibrosis, a restrictive lung disease, typically causes symptoms in the early 30's and can progress to death within a decade; it is probably caused by macrophages that constitutively produce inflammation mediators [61]. Granulomatous colitis, which occurs in 20–30% of patients with HPS1-4-6, has clinical features suggestive of UC and pathological features similar to that of CD including non-necrotizing granulomas, fissuring, and transmural inflammation. The colitis usually manifests in the first or second decades of life; it can be severe and not always responds to therapy; occasionally, colectomy may be required [61]. Although the colon is primarily involved in HPS, any part of the GI tract, including the gingiva, can be affected. GI complications are responsible for 9% of deaths [3, 13, 16, 61].

In the case of pulmonary fibrosis, death occurs during the 4th–fifth decades.

The diagnosis is based on the presence of oculo-cutaneous albinism and bleeding diathesis despite a normal number of platelets.

For HPS2, the diagnosis is based on oculo-cutaneous albinism

AND chronic neutropenia

AND at least one of the following:

- Bleeding diathesis
- Recurrent infections
- Hemophagocytic lymphohistiocytosis (HLH)

AND defective cytotoxicity caused by impaired degranulation [7]

The next step is to evaluate the absence of δ granules on freshly isolated plasma with electron microscopy. The bleeding time test is unreliable. Molecular analysis is recommended. In case of HPS type 1-2-4, high-resolution computed tomography of the chest (HRCT) is used to diagnose pulmonary fibrosis; the biopsy is not suggested due to the high risk of bleeding. Pirfenidone and lung transplantation are used against pulmonary fibrosis. Infliximab and steroids are used against GI manifestations [61, 62].

Protection from the sun is mandatory due to an increased risk of melanoma and basal-cell cancer.

Warfarin, ibuprofen, and aspirin should be avoided; single donor platelets transfusion may be used in the setting of trauma and desmopressin can be administered to prevent bleeding complications [61, 62].

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Exocrine Pancreatic Insufficiency

39

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Introduction

Exocrine pancreatic insufficiency (EPI) refers to insufficient secretion of pancreatic enzymes (acinar function) and/or sodium bicarbonate (ductal function), resulting in maldigestion and malabsorption of nutrients [1]. The symptoms and manifestations of EPI are largely due to an inability to digest fat. Symptoms are typically nonspecific but must be suspected in every child with steatorrhea (excess fat in the stool), failure to thrive of unexplained reasons, fat-soluble vitamin deficiency, as well as in children with recurrent pancreatitis [2] (see Chap. 34). References 3–29 constitute a comprehensive review of all known forms of EPI [3–29]. Table 39.1 lists both relatively common as well as rarer genetic causes of this condition.

EPI can also develop from nonpancreatic disorders [30]. In celiac disease, inflammatory bowel disease, or other conditions with proximal small-bowel mucosal inflammation, EPI can be caused by impaired release of secretin and cholecystokinin (CCK), which are potent stimulators of pancreatic secretion. Gastrointestinal surgery such as pancreatectomy, gastrectomy, small-bowel resections, or even esophagectomy may also result in EPI due to altered pancreatic enzymes and gastrointestinal hormone levels.

Exocrine Pancreatic Insufficiency in Cystic Fibrosis

Cystic fibrosis (CF) is the most common etiology of EPI in children. CF is an autosomal-recessive condition caused by defects in the cystic fibrosis transmembrane regulator (CFTR) gene. Although the severity of EPI is highly dependent on the specific genotypic mutation, about 85% of patients with CF develop EPI by 1 year of age [31].

Pathophysiology of Exocrine Pancreatic Insufficiency in Cystic Fibrosis

Mutations of the CFTR gene cause impaired chloride transport at the apical surface of epithelial cells [32] and disturb chloride-coupled bicarbonate transport [33] and sodium channel activity [34]. Pancreatic secretion of chloride, bicarbonate, sodium, and potassium in response to combined CCK and secretin stimulation is impaired in all patients with CF, regardless of pancreatic function status [35]. Bicarbonate secretion is most impaired, and defective electrolyte secretion leads to reduced fluid secretion [36]. Defective bicarbonate secretion results in impairment in the luminal flow of pancreatic enzymes and proenzymes and impairment in the trafficking of zymogen granules, leading to a severe block in acinar cell secretion followed by loss of cellular function, cell death, fibrosis, and eventual pancreatic insufficiency that leads to a decline in all the enzymes secreted by the pancreas [32, 36].

In healthy people, only 5–10% of the normal postmeal pancreatic enzyme output is adequate for normal digestion, indicating the large reserve capacity of the pancreas [32]. This reserve capacity means that clinically significant malabsorption is not evident until at least 90% of the exocrine cells of the pancreas are destroyed [37]. In normal individuals, the presence of free fatty acids in the proximal small bowel causes release of CCK, which in turn stimulates pancreatic secretion [38]. When pancreatic insufficiency begins to develop, this feedback loop is impaired and the site of maximal digestion shifts to the more distal bowel [32]. This results in larger amounts of nutrients being delivered to the distal bowel with changes in motor and secretory function of the more proximal bowel [39, 40]. These changes, in turn, lead to quicker intestinal transit and malabsorption [40].

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Table 39.1 Syndromes and genetic conditions associated with pancreatic insufficiency [3–29]

Condition	Affected gene(s)	Inheritance	OMIM	Reference
Cystic fibrosis	CFTR	AR	219,700	[3]
Shwachman–-Diamond syndrome	SBDS	AR	260,400	[4]
	DNAJC21	AR	617,052	[5]
	EFL1	AR	617,941	[6]
	SRP54	AD	618,752	[7]
Johanson–Blizzard syndrome	UBR1	AR	243,800	[8]
Pearson marrow-pancreas syndrome	Mitochondrial DNA defects		557,000	[9]
Jeune syndrome	ATD	AR	208,500	[10]
Pancreatic agenesis	PDX1	AR	260,370	[11]
	PTF1A	AR	615,935	[12]
Pancreatic agenesis and congenital heart defects	GATA6	AD	600,001	[13]
Pancreatic and cerebellar agenesis	PTF1A	AR	609,069	[14]
Congenital lipase deficiency	PNLIP	AR	614,338	[15]
Congenital enterokinase deficiency	PRSS7	AR	226,200	[16]
Hereditary pancreatitis			167,800	
Trypsin dependent	PRSS1	AD		[17]
	SPINK1	AD		[18]
	CTRC	AD		[19]
	CASR	AD		[20]
Trypsin independent	CFTR	AD		[21]
	CPA1			[22]
	CLDN2	X-linked		[23]
	MORC4	X-linked		[24]
	CELA3B	AD		[25]
Pseudohypoparathyroidism Type IA	GNAS1	AD	103,580	[26]
CoQ-responsive Oxphos deficiency	Unknown			[27]
Exocrine pancreatic insufficiency, dyserythropoietic anemia and calvarial hyperostosis	COX4I2	AR	612,714	[28]
Infantile-onset multisystem neurologic, endocrine, and pancreatic disease (IMNEPD)	PTRH2	AR	616,263	[29]

Abbreviations: *OMIM* Online Mendelian Inheritance in Man, *CFTR* cystic fibrosis transmembrane conductance regulator, *SBDS* Shwachman–Bodian–Diamond syndrome, *DNAJC21* DNAJ heat shock protein family (Hsp40) member C21, *EFL1* elongation factor like GTPase 1, *SRP54* signal recognition particle 54, *UBR1* ubiquitin protein ligase E3 component N-recognin 1, *ATD* asphyxiating thoracic dystrophy (chondroectodermal dysplasia-like syndrome), *PDX1* pancreatic and duodenal homeobox 1, *PTF1A* pancreas-associated transcription factor 1a, *GATA6* GATA-binding protein 6, *PNLIP* pancreatic lipase, *PRSS* serine protease, *SPINK1* serine peptidase inhibitor Kazal type 1, *CTRC* chymotrypsin C, *CASR* calcium-sensing receptor, *CFTR* cystic fibrosis transmembrane conductance regulator, *CPA1* carboxypeptidase A1, *CLDN2* claudin2, *MORC4* MORC family CW-type zinc finger 4, *CELA3B* chymotrypsin-like elastase 3B, *GNAS* GNAS complex locus, *COX4I2* cytochrome C oxidase subunit 4I2, *PTRH2* peptidyl-TRNA hydrolase 2, *AR* autosomal recessive, *AD* autosomal dominant

Whether a patient is pancreas sufficient (PS) or EPI has clinical and prognostic significance in CF. PS does not mean normal pancreatic function but that enough pancreatic function is present to avoid the need for pancreatic enzyme replacement therapy (PERT). Patients who are PS are more susceptible to pancreatitis [41], while EPI patients have more severe lung disease, malnutrition, and liver disease [42].

Shwachman–Diamond Syndrome

Shwachman–Diamond syndrome (SDS) is the second most common cause of EPI in children. SDS is an autosomal-recessive disorder characterized by congenital anomalies, pancreatic insufficiency, bone marrow failure, and predisposition to myelodysplasia and acute myeloid leukemia (AML) [43]. Mutations in the Shwachman–Bodian–Diamond syndrome (*SBDS*) gene on chromosome 7q11 can be found in approximately 90% of classically presenting patients with SDS [44]. The SBDS gene is involved in ribosomal function [45], and ribosomal subunit assembly is impaired in patients with SDS [46]. These mutations usually result in reduced, but not absent, protein expression. In mice, targeted deletion of the gene results in embryonic death, suggesting that some expression of this gene is necessary for survival [47]. The exact mechanism by which this leads to pancreatic insufficiency is unknown. One study found that patients negative for mutations in the SBDS gene may have more severe hematological manifestations while having milder pancreatic disease [48].

The incidence of SDS is 1:76,000 individuals with a male: female ratio of 1.7:1 [49, 50].

The classic presentation of SDS is in infancy with failure to thrive, diarrhea, and neutropenia. SDS infants have an

average birth weight at the 25th percentile [50]. Growth failure with malnutrition is common in the first year of life, and height velocity falls such that height remains below the third percentile in 38–56% of patients [51, 52]. After diagnosis, and with appropriate therapy, most children regain normal growth velocity, though height and weight remain below the third percentile [53]. Steatorrhea is caused by decreased secretion of pancreatic enzymes, while ductular fluid and electrolyte secretion of the pancreas remain normal [54, 55]. EPI tends to be diagnosed within the first 6 months of life with 90% of patients being diagnosed in the first year [54]. Spontaneous improvement in pancreatic function can occur in later childhood with 50% of patients by age 4 years having normal fat absorption and no longer requiring pancreatic enzyme supplementation [54]. The pancreas in SDS exhibits a characteristic fatty replacement, which can be visualized on ultrasound, CT, and, perhaps, best by MRI [56].

Hepatomegaly and raised serum liver enzymes are common in children with SDS [57]. These resolve by the age of 5 years, and no long-term consequences have been observed [57].

Neutropenia is the most common cytopenia and can be persistent, intermittent, or cyclic and may vary from mild to severe [50]. Anemia with low reticulocyte counts [50] and elevations in fetal hemoglobin are each seen in 80% of patients [58]. Thrombocytopenia can also be seen. Bone marrow biopsy is usually hypoplastic with increased deposition of fat [58, 59]. Patients with SDS have a propensity to developing infections due to the neutropenia and the occasional functional neutrophil deficits that are seen in SDS [60]. Patients with SDS develop clonal changes in the bone marrow, which may or may not be associated with an increased risk of myelodysplasia or acute myeloid leukemia (AML) [61]. Due to the predisposition to myelodysplasia and AML, all patients with SDS should be referred to a pediatric hematologist. Based on data from several registries, the frequency of both myelodysplasia and AML increases with increasing age [48, 62, 63]. Hematopoietic stem cell transplantation should be considered for treatment of severe pancytopenia, myelodysplasia, or AML [64].

The bony dysplasia of SDS manifests as short stature and delayed appearance but subsequent normal development of secondary ossification centers [50]. There is variable metaphyseal widening and irregularity that is most often seen in the ribs in early childhood and in femurs later in childhood and adolescence [65]. Rarely, skeletal involvement may be extremely severe and generalized [50]. Usually, these metaphyseal changes are clinically insignificant, but rarely, they may lead to limb deformities and fractures [65].

A characteristic pattern of neurocognitive and behavioral difficulties has been described in SDS [66].

A high degree of suspicion may be needed to diagnose milder cases of SDS. A study of 37 children with SDS found that neutropenia (81%), diarrhea (58%), failure to thrive (73%), lipomatous infiltration of the pancreas (~90%), low fecal elastase (82%), and skeletal (38%), congenital, and endocrine malformations (65%) were all inconsistently present [67].

Serum immunoreactive trypsinogen (IRT) and pancreatic isoamylase concentrations can be useful markers of the pancreatic phenotype in SDS [68]. In healthy children, serum IRT concentrations are at adult levels at birth, while pancreatic isoamylase concentrations are low at birth and reach adult levels by 3 years of age [68]. In contrast, in SDS, young children have low serum IRT concentrations, which then rise with age, while serum pancreatic isoamylase activities are low at all ages. Serum IRT is generally low in EPI patients with SDS, while a normal value does not rule out EPI. Serum isoamylase concentrations are not useful in determining PS or EPI. Hence, when SDS is suspected, a serum IRT should be obtained in children <3 years of age, while serum pancreatic isoamylase should be obtained in children ≥3 years of age [50].

The diagnosis of SDS is made using the criteria shown in Table 39.2. The combination of exocrine pancreatic dysfunction and hematological abnormalities when other known causes of exocrine pancreatic dysfunction and bone marrow failure are excluded gives rise to a clinical diagnosis of SDS [50]. CF should be ruled out with a sweat test, while Pearson syndrome can be differentiated by a bone marrow examination and imaging of the pancreas. Cartilage hair hypoplasia, which presents with diarrhea (but not with EPI), cytopenia, and metaphyseal chondrodysplasia, is more common in certain populations such as the Amish.

Exocrine Pancreatic Insufficiency in Chronic Pancreatitis

All children with chronic pancreatitis should be assessed for EPI at least annually and more often if symptoms develop in the interim. Children should be assessed for EPI using a stool elastase and should be considered EPI if the stool elastase <200 ug/g. EPI in children with chronic pancreatitis is managed in the same manner as EPI from any other cause.

Pearson Syndrome

Pearson syndrome (formerly Pearson Marrow-Pancreas syndrome) is a rare genetic condition with unknown prevalence. Pearson described a syndrome of refractory, transfusion-dependent sideroblastic anemia with vacuolization of the bone marrow and EPI [9]. Other variable features may include hepatic failure, proximal renal tubulopathy, watery diarrhea, patchy erythematous skin lesions, neutropenia, and thrombo-

Table 39.2 Diagnostic criteria for Shwachman–Diamond syndrome [50]

Clinical and molecular diagnostic criteria
Clinical diagnosis Fulfill the combined presence of hematological cytopenia of any given lineage (most often neutropenia) and exocrine pancreas dysfunction
Hematologic abnormalities may include the following: (a) Neutropenia <1.5 × 10^9/L on at least 2 occasions over at least 3 months (b) Hypoproductive cytopenia detected on 2 occasions over at least 3 months
Tests that support the diagnosis but require corroboration: (a) Persistent elevation of hemoglobin F (on at least 2 occasions over at least 3 months apart) (b) Persistent red blood cell macrocytosis (on at least 2 occasions over at least 3 months apart), not caused by other etiologies such as hemolysis or a nutritional deficiency
Pancreatic dysfunction may be diagnosed by the following: (a) Reduced levels of pancreatic enzymes adjusted to age [fecal elastase, serum trypsinogen, serum (iso)amylase, serum lipase]
Tests that support the diagnosis but require corroboration: (a) Abnormal 72-hr fecal fat analysis (b) Reduced levels of at least 2 fat-soluble vitamins (A, D, E, K) (c) Evidence of pancreatic lipomatosis (e.g., ultrasound, CT, MRI, or pathological examination of the pancreas by autopsy)
Additional supportive evidence of SDS may arise from the following: (a) Bone abnormalities (b) Behavioral problems (c) Presence of a first degree-family member diagnosed before with SDS
Other causes of pancreatic insufficiency should be excluded, in particular when the SBDS gene mutation analysis is negative
Molecular diagnosis: biallelic SBDS gene mutation Positive genetic testing for SBDS mutations known or predicted to be deleterious, e.g., from protein modeling or expression systems for mutant SBDS
Caveats: Many situations arise when molecular diagnosis is NOT confirmatory in the presence of clinical symptoms: No identified mutations (about 10% of cases) Mutation on one allele only Gene sequence variations that have unknown or NO phenotypic consequence A novel mutation, such as a predicted missense alteration, for which it is not yet possible to predict whether it is disease causing SBDS polymorphisms on one or both alleles. Large population studies may be needed to exclude a sequence polymorphism as a bona fide irrelevant variant

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cytopenia, and high serum lactate/pyruvate ratios [69]. This condition should be considered in the differential diagnosis of SDS. In this syndrome, vacuolization of the marrow is seen, while in SDS, the bone marrow is dysplastic; the pancreas is fibrotic in Pearson syndrome, while it is fatty in SDS [70]. Pearson syndrome is caused by large deletions or rearrangements in mitochondrial DNA, which are more abundant in the blood than in other tissues [69]. Diagnosis is suspected based on clinical findings and can be confirmed by Southern blot analysis, which detects rearrangements of mitochondrial DNA [71]. This syndrome is usually fatal in infancy, but some children who survive past infancy develop severe neurological symptoms such as proximal myopathy, seizures, ataxia, or abnormal movements, suggestive of another mitochondrial DNA disorder, Kearns-Sayre syndrome [72]. In children without multisystem involvement, bone marrow transplantation or unrelated cord blood cell transplantation has been suggested as a mechanism to manage the severe hematological manifestations of this syndrome [71].

Johanson–Blizzard Syndrome

Johanson–Blizzard syndrome (JBS) is a rare autosomal-recessive disorder caused by mutations in the *UBR1* gene on chromosome 15q15.2 [73, 74]. UBR1 encodes an E3 ubiquitin ligase that is involved in proteolysis [74]. However, the exact causative mechanism of EPI in JBS is unknown, but likely due to a near-total absence of pancreatic acini and replacement by fat. A small beak-like nose (due to aplasia or hypoplasia of the alae nasi) and EPI in early infancy are most consistently present, while other features in decreasing order of occurrence include dental anomalies, congenital scalp defects, sensorineural hearing loss, growth and psychomotor retardation, hypothyroidism, imperforate anus, and genitourinary anomalies [75].

Clinical Symptoms of Exocrine Pancreatic Insufficiency

The symptoms of fat malabsorption, which have been best described in CF, are abdominal pain, constipation, flatulence, and diarrhea [32]. Diarrheal symptoms are not different between PS and EPI patients in CF [32]. Hence, these symptoms are not good markers of EPI and definitely not measures of adequacy of PERT.

EPI can result in significant malnutrition and nutritional deficiencies, particularly of fat-soluble vitamins A, D, E, and K [76]. Zinc, iron, calcium, folic acid, magnesium, and selenium deficiencies have been described in CF [76, 77]. In CF, the presence of liver disease and enteropathic changes may create further nutritional issues.

Diagnosis of Exocrine Pancreatic Insufficiency

The ideal test of pancreatic function should be specific, non-invasive, able to quantitate pancreatic function, able to indicate the need and the appropriate dosage of substitutive enzymes even during therapy, cost-effective, and broadly available [78]. No such test is presently available [78]. Tests

Table 39.3 Exocrine pancreatic function tests

Direct tests	Indirect tests
Nonstimulatory test Fecal elastase-1 Fecal chymotrypsin Immunoreactive trypsinogen (IRT), lipase, and isoamylase levels	Spot fecal fat[a] Steatocrit[a] 72-hour fecal fat excretion test ^{13}C-labeled mixed-triglyceride breath test
Stimulatory test Secretin stimulation test Cholecystokinin stimulation test Secretin-cholecystokinin stimulation test	

[a]Not recommended

for EPI can be performed by measuring pancreatic secretion (direct tests) or by estimating the consequences of malabsorption (indirect tests) [1]. Common tests for EPI are summarized in Table 39.3.

Indirect Pancreatic Function Tests

The standard indirect pancreatic function test measurement of fat absorption is the 72-hour fecal fat excretion test. The coefficient of fat absorption (CFA) is calculated as follows:

((Fat ingestion – Fat excretion) / Fat ingestion) × 100(%). Normal CFA values are >85% in infants <6 months of age, and >95% in older children. During this time, stool is collected while consuming a standardized high-fat diet or more typically ingestion is recorded in a detailed food diary [79]. Microscopic examination of a spot stool sample using Sudan stain to detect fat droplets or the acid steatocrit is not recommended due to their lack of sensitivity and specificity.

The ^{13}C-labeled mixed-triglyceride breath test (^{13}C -MTG) is a noninvasive method of assessing lipase activity [80, 81]. Orally administered ^{13}C-labeled fatty substrates are digested by the pancreatic lipase; the released free fatty acids or monoglycerides are absorbed in the gut and oxidized in the liver to $^{13}CO_2$, which is rapidly exhaled in breath and can be measured. Several studies have shown a good correlation of ^{13}C -MTG and fecal fat quantification, with high sensitivity and specificity for the diagnosis of EPI [82, 83]. ^{13}C -MTG can also be used to assess the efficacy of PERT.

The disadvantages are the unavailability of the test in the United States, variability of test protocol, and the age limit as younger patients may not be able to follow the testing instructions. The test results can be influenced by other conditions such as liver disease, gastrointestinal dysmotility, and lung disease.

Direct Pancreatic Function Tests

Fecal elastase-1 is the most commonly used test to diagnose EPI. The test, although called fecal elastase-1, does not actually measure elastase-1 as this is transcriptionally silenced in humans. The test actually measures chymotrypsin-like elastases (CELA) 3A and 3B [77]. These enzymes are secreted by the pancreas and do not undergo degradation in the gut. The monoclonal fecal elastase-1 test only identifies the human form of the enzymes; hence, the test can be done even when the patient is receiving porcine-derived PERT [84]. It is inexpensive with good sensitivity and specificity for detection of EPI. The sample needs a very small amount of stool (≥1 gram) and is stable for weeks at room temperature [85]. Falsely low values may be obtained when the stool is dilute as in any diarrheal illness or in the presence of enteropathies.

After 2 weeks of age, PS patients can be differentiated from EPI patients using a cut-off of 200 μg/gram [86], whereas others have suggested that 180 μg/gram is a more accurate cut point in patients with CF [87]. Fecal elastase-1 can also be used in the annual monitoring of CF patients with PS to identify the onset of EPI.

In patients with CF, the presence of a low fecal elastase-1 is usually considered diagnostic of EPI despite the fact that fecal elastase-1 correlates poorly with fecal fat excretion [88]. In other conditions, especially in children who present with poor growth or malabsorption, the next steps after obtaining a fecal elastase-1 level are less clear. Also, preliminary reports suggest that drugs regulating CFTR (e.g., ivacaftor) may increase fecal elastase-1 and potentially convert children with EPI to PS; however, more data are needed to confirm these findings [89–92].

The CF Foundation recommends an evaluation of pancreatic functional status by fecal elastase or coefficient of fat absorption for all children with CF under 2 years of age [93]. Fecal elastase should be repeated at age 1 year in children diagnosed with EPI in infancy, especially in those with an initial fecal elastase value of >50 μg/gram to ensure that those with a falsely low fecal elastase value do not receive PERT unnecessarily [85].

Fecal chymotrypsin is a less sensitive and specific marker of pancreatic function than fecal elastase-1. However, this test, which does identify porcine enzymes, can be used to monitor compliance to PERT in patients who are known to be EPI [94].

Serum IRT of less than 20 ng/mL is specific for EPI [95]. Serum IRT levels, as a part of newborn screen, are at adult levels at birth in healthy newborns, but markedly elevated in newborns with CF regardless of whether their mutation is pancreatic sufficient or insufficient [96, 97]. Serum IRT levels are generally reduced later in life in patients with EPI, but a normal value does not rule out EPI.

Stimulatory tests or direct pancreatic function tests using secretagogues such as CCK and/or secretin are currently considered the gold standard to evaluate EPI. Unlike indirect pancreatic function tests, this assay can evaluate contents of pancreatic fluids and differentiate EPI from specific pancreatic enzyme deficiencies.

The original test performed by using Dreiling tubes was time-consuming and poorly tolerated by patients, so it has been replaced by an endoscopic method [98, 99]. Either secretin or CCK is administered intravenously prior to endoscopic intubation. Within 10 minutes, pancreatic fluid is collected from the duodenum (close to the ampulla of Vater). The pancreatic fluid can be evaluated for pH, protein content, amylase, lipase, trypsin, chymotrypsin, elastase, and electrolytes including bicarbonate [100].

The pancreatic stimulation test appears to be one of the most sensitive tests for the diagnosis of severe chronic pancreatitis and EPI, but it is less sensitive in less advanced disease. False abnormal results may be reported in patients with diabetes, celiac sprue, advanced liver disease, or those with a recent episode of acute pancreatitis [101].

Pancreatic stimulation testing may be performed with the use of a magnetic resonance cholangiopancreatography (MR PFT) to help assess pancreatic anatomy and quantify exocrine pancreatic function. Adult data support the use of MR PFT as a noninvasive and radiation-free method. However, a standardized protocol with an exact interpretation of findings needs to be developed for use in children [102, 103].

Management

Much of the data on management of pancreatic insufficiency in children is extrapolated from CF. In general, the symptoms of pancreatic insufficiency can be more easily controlled in conditions other than CF than in CF. PERT remains the mainstay of treatment for patients with clinical symptoms of pancreatic insufficiency or laboratory signs of malabsorption in CF and non-CF EPI [104]. In CF, PERT is indicated in all infants with two CFTR mutations associated with EPI [93]. FDA-approved PERT are summarized in Table 39.4. All PERT products are of porcine origin and con-

Table 39.4 FDA-approved pancreatic enzyme products

	Dosages available (lipase/protease/amylase units)	Bead/ microsphere diameter (mm)	Notes
Creon ®	3000/9500/15,000 6000/19,000/30,000 12,000/38,000/60,000 24,000/76,000/120,000 36,000/114,000/180,000	0.7–1.6	Oral, delayed release capsules
Pancreaze ®	2600/6200/10,850 4200/14,200/24,600 10,500/35,500/61,500 16,800/56,800/98,400 21,000/54,700/83,900	2	Oral, delayed release capsules
Pertyze ®	4000/15,125/14,375 8000/28,750/30,250 16,000/57,500/60,500 24,000/86,250/90,750	0.8–1.4 for 4000; 0.8–2.2 for others	Oral, delayed release capsules with bicarbonate-buffered enteric-coated microspheres
Ultresa ®	13,800/27,600/27,600 20,700/41,400/41,400 23,000/46,000/46,000	2.0–2.4	Oral, delayed release capsules approved for use in >12 months plus a weight requirement
Viokace ®	10,440/39,150/39,150 20,880/78,300/78,300	N/A	Nonenteric-coated tablets Approved only for use in adults Must be given with a proton pump inhibitor
Zenpep ®	3000/10,000/14,000 5000/17,000/24,000 10,000/32,000/42,000 15,000/63,000/47,000 20,000/63,000/84,000 25,000/79,000/105,000 40,000/126,000/168,000	1.8–1.9 for 3000 and 5000; 2.2–2.5 for others	Oral, delayed release capsules
Relizorb ®	Only contains lipase 1 cartridge per 500 mL of enteral formula, up to 2 cartridges per day	Unknown	Use with soluble fiber containing enteral feeding only

Creon® [package insert]. North Chicago, IL: Abbott Laboratories; 2020
Pancreaze® [package insert]. Titusville, NJ: Janssen Pharmaceuticals Inc.; 2018
Pertyze® [package insert]. Bethlehem, PA: Digestive Care Inc.; 2017
Ultresa® [package insert]. Birmingham, AL: Aptalis Pharma US Inc.; 2012
Viokace® [package insert]. Birmingham, AL: Aptalis Pharma US Inc.; 2012
Zenpep® [package insert]. Madison, NJ: Allergan Therapeutics LLC.; 2020
Relizorb® [package insert]. Newton, MA: Alcresta Inc.; 2014

tain a mixture of the digestive enzymes, lipase, protease, and amylase in varying proportions. However, because lipase plays the main role in therapy, PERT dosage is based on the content of lipase units [104].

Enzyme dosing can be based on body weight (units of lipase/kg per meal) or the amount of fat present in the food (units of lipase/grams of fat eaten), but dosing based on body weight is more practical. In infants with CF, PERT doses are generally 2000–4000 lipase units per 120 mL of formula or per breast-feeding. In all other patients with EPI, the dose is gradually adjusted based on weight gain and absorption to a maximum of 2500 lipase units per kilogram per meal (not to exceed 10,000 lipase units/kilogram per day or 4000 units lipase/ gram dietary fat per day) [105]. Higher doses have been associated with fibrosing colonopathy and are not recommended [106]. Other side effects of PERT include soreness in the mouth and perianal irritation [107]. Allergies may occur due to the porcine origin of the enzyme preparations [107]. Hyperuricemia which was seen with older preparations is rarely seen now [108]. A sudden introduction of PERT to patients with uncontrolled fat malabsorption may lead to severe constipation with accompanying abdominal pain [107].

Enteric-coated microspheres or mini-microspheres of <2 mm in size are the preparations of choice for PERT. The efficacy data of micro- or minitablets of 2.2–2.5 mm in size is limited. In infants, the enzyme microspheres are mixed with a small amount of breast milk or infant formula or soft, acidic food with a pH of less than 4.5 such as applesauce and given via spoon immediately before the feed. In older children, the enzyme should be distributed to a full dose with main meals, and half that dose with snacks. Enzymes should either be given at the beginning of the meal or given half at the beginning and half midway through the meal, particularly if the feeding time is longer than half an hour. The capsules should be swallowed whole without crushing or chewing at as early an age as possible. In a tube-fed patient, PERT in a cartridge form can be connected directly to the enteral feeding pump.

Dietary fat restriction and very high-fiber diets should be avoided in children in EPI (CF and non-CF). Small, frequent, high-energy meals are recommended. However, some children with poor growth, particularly with CF, may not respond to high-calorie foods and oral high-calorie beverages. These children may be candidates for supplemental nocturnal gastrostomy tube feeding. In these patients, pancreatic enzymes should be given before and after the tube feeding or a digestive-enzyme cartridge that can be connected to the enteral feeding system can be used and has been shown to be effective in children with CF. While a polymeric formula is typically used, some children may require a protein hydrolysate formula due to uncontrolled malabsorption or poor growth despite adequate caloric intake. In children receiving the maximum dose of PERT, additional benefit may be obtained by the addition of zinc supplementation (1 mg elemental zinc/kg per day) [93] or acid-blockade medications (proton pump inhibitor) to prevent deactivation of enzymes by reducing luminal acidity. Other causes of malabsorption should be considered if no improvement is observed with all these strategies.

The efficacy of PERT can be observed by the improvement of malabsorptive symptoms such as steatorrhea, bloating, or poor weight gain and the nutritional status of the patients. However, there is no correlation between PERT dose and symptoms [32]. If no improvement is observed, a repeat quantitative fecal fat estimation with PERT or fecal chymotrypsin might be helpful.

In children with CF, CF-specific vitamin preparations are recommended. These vitamin preparations as well as the regular assessments of vitamin status should be considered in all children with pancreatic insufficiency. All patients with EPI should have measurement of fat-soluble vitamins (A, D, E, and K) at diagnosis and then every 6–12 months or every 3 months after a change in vitamin therapy [109]. It is recommended that these vitamins be taken with a fat-containing meal along with PERT. Monitoring of other vitamins, minerals, or trace elements is not routinely recommended unless deficiencies are clinically suspected.

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Celiac Disease

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Introduction

Celiac disease (CD) is a complex autoimmune disorder elicited by the ingestion of gluten (the main storage protein in wheat, barley, and rye) in genetically predisposed individuals expressing specific human leukocyte antigen (HLA)-class II haplotypes (called DQ2 and DQ8) and causing an inflammatory disorder of the small intestine. CD is characterized by a variable combination of elevated titers of celiac-specific autoantibodies, an inflammatory enteropathy with variable degrees of severity, and a gastrointestinal (GI) as well as a wide range of extra intestinal complaints [1] whose severity varies from asymptomatic to life-threatening. CD is well known to be often associated with other autoimmune conditions such as type 1 diabetes and autoimmune thyroiditis [2], as well as with some congenital disorders such as Down [3, 4] Turner [5], and most recently also Williams-Beuren syndrome [6].

Epidemiology

Currently, it is estimated that CD has a global prevalence around 1% of the general population [7], although there are important variations between different geographical areas [8–10], even within a single country [11]. Furthermore, CD is more prevalent in females than males [7]. However, since studies of prevalence based on screening do not show such clear-cut difference, it is currently thought that the female predominance could also in part be attributed to the fact that men appear to underutilize health-care services [12, 13]. Over the past several years, it has also become clear that CD prevalence has been progressively increasing [9, 14–18]. While the availability of reliable serological tests to screen for CD as well as an increased awareness, especially in North America, can be considered responsible for increased diagnostic rates, reliable epidemiological data convincingly show worldwide a true increase in prevalence, of the order of doubling rates every 20 years or so. In Northern Sweden, an epidemiological investigation employing a combined serological/endoscopic approach in an unselected population of 1000 adults found a prevalence of almost 2% [19]. Since obviously genetic changes cannot be blamed for this rapid change, a variety of environmental factors have been called as potentially responsible for such increase, but their respective role is still unclear [20]. In fact, for some suspected factors the evidence is at best equivocal or contradictory, among them newer varieties of wheat obtained by breeding techniques [21–23], seasonality and modalities of delivery [24–28], and exposure to antibiotics [29–33]. On the other hand, infections in early life [34, 35] and especially respiratory infections [36] including influenza [37] and infections by *Enterovirus* [38] appear likely to increase the risk of CD development. In this respect, the finding of the role of reovirus in inducing inflammatory response to dietary antigens and favoring CD development [39] appears a key finding. Interestingly, vaccinations do not represent a risk factor [40].

Infant feeding practices have also been deeply scrutinized in a number of observational and interventional studies, and their results can currently be summarized as follows [41, 42]:

- Breast feeding, long thought to have a protective effect on the development of CD, appears not to be beneficial in this regard [43–46].
- Avoidance of cow's milk in early life (suggested to reduce the risk of CD later in life in children at genetic risk) has no protective effect [47].

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- Early (before the end of the third month) introduction of gluten is not recommended as it is considered a risk factor [48]. In contrast to this, however, a recent prospective study in the UK [49] showed a different scenario, suggesting on the contrary that – similar to some food allergies – early introduction of gluten could be associated with a *reduced* rate of subsequent CD. As it can be seen therefore, the field is still a rapidly evolving one.
- Introducing gluten in small amounts [44] or after the first year of life [43] does not offer protection, as the strongest factors favoring the onset of CD appear to be gender and HLA status.
- Intake of large amounts of gluten during the first 2 years of age increases the risk of CD in genetically susceptible children [45].
- The possible role of maternal diet during pregnancy and on diet of the child after weaning in influencing the onset of CD has also been investigated. A recent study on more than 80,000 participants [50] showed that higher maternal fiber intake (median 29.5 g/day) was associated with a lower risk of CD in the offspring, and that gluten intake during pregnancy (median 13.0 g/d) was associated with a higher risk of childhood CD, suggesting that maternal diet during pregnancy could impact later CD autoimmunity [50].
- In a large prospective study of dietary patterns of young children, Barroso et al. [51] found that high consumption of vegetables and grains and low consumption of refined cereals and sweet beverages are associated with lower odds of developing CD autoimmunity, the first step in the development of the disease. Thus, an eating pattern that can be reasonably liked to a Mediterranean diet, as opposed to the "Western" diet that appears to be linked with various chronic inflammatory disorders, may have a protective effect.

A plausible mediator of many of the environmental factors mentioned is therefore the microbiome, whose composition and modifications have been shown to play a critical role in the onset (or prevention) of allergic and inflammatory disorders. In recent years there has been therefore an increasing focus on the role of microbiota in the development of CD [52] (see below).

Etiopathogenesis

Like most multifactorial disorders, CD is the result of a complex interaction between genes and immune status of the host and environmental triggers.

Genetic Factors

The weight of genetic predisposing factors in CD pathogenesis is relevant, as initially suggested by the observation of a strong familiar aggregation [53], with a prevalence of CD among first-degree relatives of around 10%. The most important and best-characterized susceptibility gene is the HLA class II; in particular, the HLA-DQ2 and HLA-DQ8 alleles are required for CD development. The concordance rate is about 30% among HLA-identical siblings, 80% in monozygotic twins, and 10% in dizygotic twins [54]. The HLA-DQ2 molecules are expressed on the surface of antigen-presenting cells and contain positively charged pockets that preferentially bind negatively charged epitopes, such as deamidated gliadin peptides (DGP), and present them to cluster of differentiation (CD)-4$^+$ T cells, thereby activating them [55, 56].

The Central Role of HLA-DQ Haplotype

HLA genes are located on chromosome 6 and are divided into three classes (I–III). HLA-DQ (6p21.3) belongs to class II and is composed of a heterodimer located on antigen-presenting cells and encoded by HLA-DQA1 and HLA-DQB1. Each of the copies of the HLA-DQ gene encodes for a heterodimer, resulting in four proteins (one couple per chromosome). HLA-DQ2 homozygote (DQB1*02 on both chromosomes) subjects have the highest risk to develop CD, fivefold higher than heterozygotes [57, 58]. Even if rarer in the general population, DQ2 homozygosis is characteristic of 25% of all CD patients and often relates to a more severe clinical outcome, as suggested by its association with earlier disease onset [59, 60] and its higher prevalence in patients with refractory CD [61], a rare but severe complication of CD. As a matter of fact, the immune response arising in homozygous individuals is stronger than the response in heterozygous individuals [53, 62]. The most common configuration (more than 50% of CD patients) is represented by DQ2.5 (DQB1*02/DQA1*05) heterozygotes [58]. HLA-DQ8 is found in 5–10% of CD patients [58, 63], with HLA-DQ8 homozygosis conferring increased risk compared to HLA-DQ8 heterozygosis [64]. It must be noted that about 5% of CD patients carry only one of the HLA-DQ2 heterodimer alleles (HLA-DQA1*05 or HLA-DQB1*02), therefore encoding for the so-called half heterodimer. These patients constitute the overwhelming majority of CD subjects not carrying the full HLA-DQ2 and/or -DQ8 alleles [65]. The highest risk group includes individuals who inherit both DQ8 and DQ2. Only less than 1% of patients who fulfill clinical criteria for CD do not carry the DQ2 (including half heterodimer) nor the DQ8 alleles [65]. Thus, in the clinical practice, CD can be virtually excluded in individuals lacking

HLA-DQ2 or HLA-DQ8. Interestingly, 30% of European descent individuals carry HLA-DQ2 susceptibility allele; however, only about 4% of these individuals will develop CD [57]. This suggests that even though those molecules are necessary, they are not sufficient to induce the disease, implying that other genetic and environmental factors must contribute to it.

Non-HLA Genetic Susceptibility Factors

HLA alleles explain 35% of the disease genetic susceptibility, while the remaining is due to numerous non-HLA genes that singularly contribute to a much lower extent to CD heritability. Genome-wide association studies (GWAS) performed over the years identified 40 CD-associated susceptibility loci (including the HLA locus) [66]. More recently a meta-analysis identified 2 additional non-HLA CD risk loci reaching genome-wide significance, bringing the number up to 41 [67]. Notably, 64% of the non-HLA loci were shared with at least another autoimmune disease (e.g., type 1 diabetes), reinforcing the idea that autoimmune disorders may share common pathogenic pathways [68]. In those regions, more than 115 non-HLA genes have been associated to CD, individually contributing only modestly to the overall disease risk. Twenty-eight of them encode for molecules involved in the immune response, reinforcing the central role of immune dysregulation in CD pathogenesis. Post-GWAS will need to focus on elucidating the functional basis of these genetic variants and in particular the role of regulatory variation. Furthermore, considering multiple gene variants may help refine risk prediction models and identify high-risk individuals that could benefit from preventive strategies.

Environmental Factors

The high impact of genetic predisposing factors in CD does not exclude a key role of the environment in triggering disease development. In fact, as mentioned above, the increased incidence of CD in the past decades cannot be explained by genetic drift and most likely results from faster changes in the environment. In an elegant review, Abadie et al. [69] showed that CD prevalence does not perfectly correlate, as it would be predicted, with the levels of wheat consumption and frequencies of HLA-DQ predisposing alleles, thus emphasizing the role of environmental factors in CD onset.

The role of infant feeding practices on CD development has been discussed above. Below, we will discuss the role of viral infections and intestinal microbiota.

Viral Infections

Infective agents have been investigated as environmental factors that contribute to trigger CD, as suggested by both epidemiological and genetic studies. GWAS identified polymorphisms in genes involved in the response to viral infections to be associated with CD. Higher rates of summer births were described in children with CD, suggesting that exposure of 4–6-month-old infants to winter-linked viral infections such as rotavirus may play a role. The first correlation between viruses and CD dates back to the 1980s when a homology between alpha gliadin and a protein of the human adenovirus [70] had been described and related to increased frequency of adenovirus infection in CD patients [71] compared to controls. Increased anti-rotavirus antibody titers have also been associated with a moderate, but significantly increased risk of CD in HLA-susceptible children [72]; subsequently [73] anti-rotavirus antibodies have been found related with CD but not with type 1 diabetes mellitus (T1DM) onset, despite the common genetic background and similar pathogenic mechanisms. Over the past decade, several publications have explored the association between viral infections and CD development. Overall, they reported that a high number of infections in the first months of life are associated with an increased risk of later CD development [74]. Of note, the risk increased synergistically if, in addition to infectious episodes, dietary gluten was first introduced in large amounts after breastfeeding was discontinued [35]. Respiratory infections in particular [37, 75] appear to be the ones most commonly associated with CD. Importantly, the risk variance explained by respiratory infections was higher than that explained by sex or HLA [36]. And recently, a clear role for *Enterovirus* infections in early life has also been reported [38]. Altogether these observations provide a strong support for the role of viral infections in CD development. Nevertheless, despite viral infections being very common and HLA-DQ2/8 being present in about 40% of the general population, only 1% develops CD, raising questions about the pathogenic mechanisms behind this association. The first work showing evidence for a mechanism behind the association of viral infections and CD was published in 2017 and showed that a specific strain of reovirus (T1L), despite being cleared by the host, could disrupt immune homeostasis at intestinal sites of oral tolerance by suppressing peripheral regulatory T cell conversion and promoting a proinflammatory T helper cell response to orally ingested gluten [39]. Importantly, the two responses depended on distinct but interplaying pathways. Lastly this study showed increased anti-reovirus antibodies in CD patients, suggesting that the risk of later disease development is independent from the severity or virulence of the infection; in fact reovirus causes asymptomatic infections in humans. Whether there is an early time window during which infections are more likely to promote CD, remains to be determined, as well as the interplay with other factors such as timing and amount of first dietary gluten introduction, breastfeeding, and declining of maternal antibody titers.

Finally, the role of viral infections beyond promoting loss of tolerance to gluten, such as their ability to enhance the progression of the disease to tissue damage, is unexplored.

Intestinal Microbiota

The composition of intestinal microbiota as well as bacterial metabolic products can affect intestinal epithelium function and mucosal immune homeostasis. Both quantitative and qualitative differences in the composition of the intestinal microbiome have been observed in patients with autoimmune disorders [76, 77], as well as CD, as recently reviewed [52] when compared to healthy individuals. In addition, disease activity and response to the diet may also play a role [78].

The variability between fecal and duodenal microbial niches, the different methodologies used across studies, and the heterogeneity of CD patients make the identification of a CD-specific microbiota a hard task. Nevertheless, most evidences point to an expansion of Proteobacteria and opportunistic pathogens such as *Neisseria* [79] or *Escherichia coli*, as well as higher bacterial virulent genes, supporting the idea of a shift toward a proinflammatory microbial community [78].

Even when looking at children at higher genetic risk to develop CD, an increase in pathogenic bacteria has been observed [80]. In fact, the HLA genotype seems to influence the early intestinal microbial composition [81]. Moreover, non-HLA genes associated with protection to infections or bacterial recognition have been related to CD, suggesting that a genetically determined pattern of microbial colonization might influence the immune maturation process and thus promote later CD onset [82].

Currently, it is still unclear whether the observed changes in the microbial composition are the cause or the result of the intestinal inflammatory process. Indeed, epithelial alterations, such as those described in CD, could create a specific environment favoring the selective colonization of some microbial species, thus contributing to the creation of the microenvironmental milieu that favors the disease development. This is clearly a rapidly evolving field, likely to bring important new acquisitions in the near future.

Pathogenesis

CD is a chronic inflammatory disorder with autoimmune features, characterized by a gluten-specific T cell-mediated immune response arising in the small intestinal mucosa upon gluten ingestion. In genetically susceptible individuals, gliadin peptides activate stress pathways and provide ligands for innate immune receptors, leading to the release of proinflammatory mediators that then sustain the T cell response both in the lamina propria and in the epithelium (Fig. 40.1). This inflammatory reaction typically involves both the innate and the adaptive arms of the immune response [83]. In this model, two hits are required, each one being necessary but not sufficient for the development of CD: one is the activation of stress markers in intestinal epithelial cells and the second is the rise of a gluten-specific proinflammatory $CD4^+$ T cell response in the lamina propria. These two events lead to the full activation of lymphocytes in the intestinal epithelium (intraepithelial lymphocytes (IELs)), final responsible of tissue destruction [84, 85].The major players of the pathogenic events leading to full-blown CD are illustrated in the following sections.

Gluten

The immunological reaction occurring in CD patients follows the ingestion of gluten-containing cereals: wheat, rye, and barley. Glutenins and gliadins, typical gluten components, are responsible for the viscosity and elasticity of the wheat dough [86]. Their high concentration of glutamine and proline residues (35% and 15% of the total amino acid content) renders them highly resistant to gastrointestinal enzymes. Indeed, the lack of prolyl-endopeptidase activity in any of the human digestive enzymes prevents enzymatic attack of proline-rich domains in gluten proteins. Thus, at the end of a normal, full digestive process of gluten, many gliadin peptides remain undigested in the intestinal lumen. The mechanism through which such peptides reach the intestinal lamina propria is not entirely clear. Gliadin is able to enhance gut paracellular permeability via zonulin-mediated pathway, a molecule involved in tight junction disassembly, as suggested also by genetic studies associating tight junction genes with CD [87]. Moreover, there is evidence that gluten can cross the intestinal epithelium through the transcellular pathway once tolerance to gluten has been broken.

Even though an increase in intestinal permeability has been shown in CD, a retro-transcytosis pathway has been described for secretory antibodies, potentially having a role also for gliadin transport. In fact, the transferrin receptor CD71, normally expressed on the basolateral side of enterocytes, is overexpressed on the luminal side of the intestinal epithelium in active CD patients, leading to an apical-to-basal retro-transcytosis of gliadin peptides complexed with secretory IgA [88]. This process protects gliadin fragments from degradation and facilitates their access to the intestinal lamina propria, thereby perpetuating the inflammatory process.

Tissue Transglutaminase 2 and Autoantibodies

In the lamina propria, tissue transglutaminase 2 (tTG), a calcium-dependent transamidating enzyme, mediates the conversion of glutamine residues into glutamic acid, introducing negatively charged residues into gliadin peptides that act as immunogenic epitopes binding to HLA-DQ molecules with relatively higher affinity, thus representing a pre-

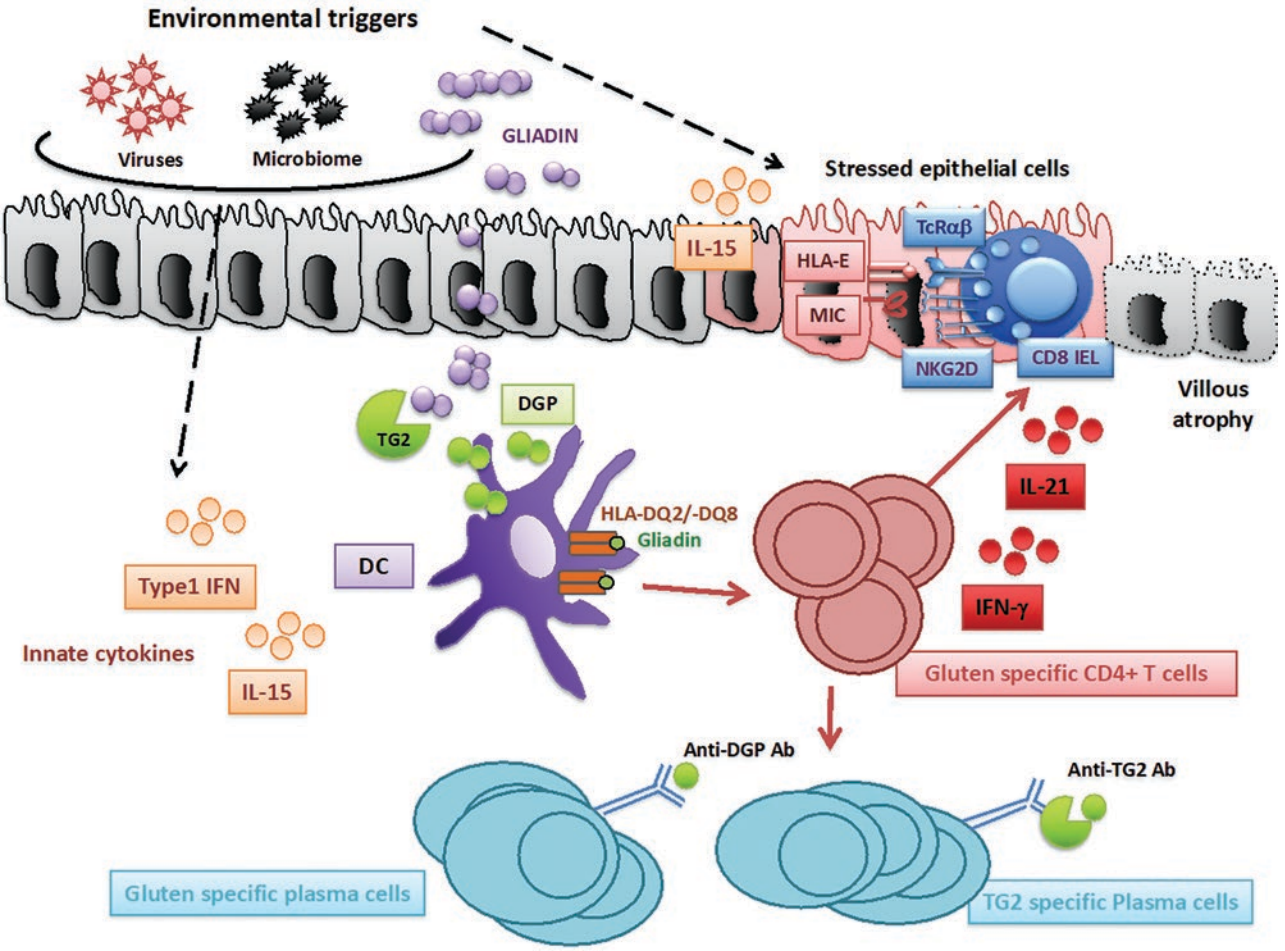

Fig. 40.1 CD pathogenesis. Celiac disease (*CD*) is a multifactorial disorder induced by gluten in genetically susceptible subjects. Several environmental factors (i.e., viral infections, alterations in microbiome composition, etc.) can contribute to trigger the disease by inducing epithelial stress in the intestinal mucosa, and the production of innate immune cytokines, such as interleukin (*IL*)-*15* and type-1 interferon (*IFN*). Those cytokines contribute to create a proinflammatory environment that enhances dendritic cell (DC) activation. Undigested gliadin peptides (*purple circles*) reach the intestinal lamina propria where they are deamidated by tissue transglutaminase 2 (*TG2*). Deamidated gliadin peptides (DGP) are more suitable to be presented by HLA-DQ molecules. Only DCs expressing the CD-associated HLA-DQ2 and/or HLA-DQ8 molecules (*orange*) can present deamidated gliadin peptides (*green circles*) to naïve CD4⁺ T cells (*red cells*). Inflammatory DCs (*purple*) enhance the rise of a gluten-specific CD4⁺ T cell response, characterized by the production of high levels of proinflammatory cytokines such as *IFN-γ* and *IL-21*. Furthermore, gluten-reactive CD4⁺ T cells provide the required help to TG2-specific B cells, presenting TG2-gliadin complexes, in a hapten carrier-like manner, and drive TG2-specific antibody production by plasma cells (*light blue cells*). IL-15 and IL-21 contribute to the full activation of cytotoxic CD8⁺ intraepithelial lymphocytes (*IEL*). An increased epithelial expression of stress markers (i.e., MIC, HLA-E, and IL-15) is also required to enable activated CD8⁺ IEL to target the intestinal epithelial cells and determine villous atrophy in CD patients. NKG2D natural killer group 2, member D, MIC macrophage inhibitory cytokine

requisite for a gluten-specific T cell response [89]. In addition to its crucial role in inducing post-translational modification of gluten peptides, tTG is also the main autoantigen for CD. In fact, increased serum levels of anti-tTG IgA (or IgG, in case of IgA deficiency) autoantibodies is a key diagnostic feature of CD. Their production seems to occur at the level of the small intestine. The most accepted hypothesis for their formation is that the enzyme tTG cross-links itself to gluten during the substrate-enzyme interaction [90]. Once internalized, those complexes are processed and gluten epitopes bind HLA-DQ molecules of the B cell that may allow gluten-reactive CD4⁺ T cells provide the required help to tTG-specific B cells in a hapten carrier-like manner and drive tTG-specific antibody production. In fact, a very high proportion of the gut plasma cells, which expand massively in the lamina propria during active CD, were shown to produce IgA specific for gluten or TG2 or both.

The Intersection of Adaptive and Innate Immune Responses in CD

Gluten contains a large number of peptides capable of stimulating T cells [91]. Dendritic cells present negatively charged glia-

din peptides through HLA-DQ2 or HLA-DQ8 molecules to naïve CD4+ T cells, thus enhancing a gluten-specific T helper 1 (Th1) inflammatory response, characterized by the production of high levels of interferon gamma (IFN-γ) and interleukin (IL)-21 in the small intestinal lamina propria (Fig. 40.1). The gluten-specific CD4+ T cell response, which contributes also to the autoantibody's generation, is sustained by several cytokines, including IL-15 and IL-21, that synergistically promote gluten-mediated increase of IFN-γ production. The expression of IL-21, enhanced by gluten, is very high in active CD patients, while it is downregulated in potential CD [92], suggesting that this cytokine plays a key role in the development of tissue damage. The rise of a gluten-specific CD4+ T cell response could be triggered by specific viral infections, as shown for reovirus T1L strain that can induce loss of tolerance to oral antigens via an IRF-1-mediated pathway [39], but also possibly by bacterial antigens. Once activated, the gluten-specific T cell response contributes to amplify the inflammatory response; nevertheless, as evident in potential CD patients, the gluten-specific T cell adaptive immune response alone can occur even in the absence of villous atrophy, suggesting that other signals are required to induce tissue damage.

In addition to the immunodominant epitopes, which, as discussed, are presented to CD4 naïve T cells, gliadin also contains fragments which are able to enhance epithelial stress and induce innate immune effects [93]. The most studied fragment is the peptide 31–43 (P31–43) of α-gliadin, which is able to upregulate major histocompatibility complex (MHC) class I-related molecules (MICs) [94], to activate the mitogen-activated protein (MAP) kinase pathway and induce apoptosis in intestinal epithelial cells. P31–43 induces cell proliferation and actin cytoskeleton rearrangements in *in vitro* and *ex vivo* models [95, 96]. The proliferative response elicited by P31–43 in CD mucosa involves epidermal growth factor (EGF)/IL-15 cooperation. In addition, P31–43 enhances the expression of IL-15 in the small intestinal epithelium. IL-15 contributes to enhance the expression of activating natural killer receptors (NKRs) on IELs and impair the function of regulatory T cells in CD patients [97]. Activating NKRs are required for the acquisition of cytolytic property by IELs. In addition to IL-15, type-1 IFNs, innate cytokines promoted upon viral infections, are responsible for inhibiting the regulatory T cell response that counterbalance the proinflammatory T cells [39]. The interplay between the adaptive gluten-specific T cell response and the innate immune pathways is key to determine the full activation of IELs that finally leads to villous atrophy.

IELs Activation and the Induction of Tissue Damage

The profound remodeling of the small intestinal architecture typical of CD is characterized by intraepithelial lymphocytosis, villous atrophy, and crypt hyperplasia. IELs represent a heterogeneous population including TcRα/β CD8 cells, NK-like cells, and TcRγ/δ cells. An increase in both TcRα/β and TcRγ/δ populations has been described in CD patients; however, the role of the former has been investigated more extensively than the latter in the context of CD pathogenesis. A permanent reshaping of the TcRγ/δ IELs compartment has been recently described in CD as characterized by an expansion of gluten-sensitive and IFN-γ-producing Vδ1+ IELs that the exclusion of dietary gluten was insufficient to revert [98].

TcRα/β IELs are the final responsible for tissue destruction. A fully active phenotype characterized by an upregulation of activating NKRs, downregulation of inhibitory NKRs, and expression of granzyme B and perforin is typically found in active CD patients [84]. The activation of their cytotoxic properties requires also stimuli from the epithelial compartment. An increased expression of stress molecules, such as nonclassical MHC class I molecules (i.e., MIC and HLA-E), has been found in intestinal epithelial cells of active CD patients, as part of the innate stress response induced by gluten or by other environmental triggers. Notably, MIC and HLA-E are ligands for NKG2D and CD94, respectively, activating NKRs that are upregulated on IELs in active CD patients and whose expression is enhanced by IL-15 [83–85].

Increased levels of both IL-15 and IL-21 in the duodenal mucosa of untreated CD patients cooperatively promote the activation of TcRα/β IELs by increasing their transcriptional, proliferative, and cytolytic activities [99]. The evidence that low levels of both cytokines are found in potential CD patients supports their key role in tissue damage. The activation of IELs, with increased Fas ligand expression, results in epithelial cell apoptosis and villous atrophy via interactions with Fas on intestinal epithelial cells.

The end result of the above-described immune events is the typical CD lesion characterized by intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy. These changes occur in a continuum: from a normal mucosa to a complete flattening of the villi in a slow progression. Marsh described in detail such progression [100] and his scoring system is utilized by pathologists to classify CD duodenal damage as follows:

Type 0 or pre-infiltrative stage (normal)
Type 1 or infiltrative stage (increased IELs)
Type 2 or hyperplastic stage (type 1 + hyperplastic crypts)
Type 3 or destructive stage (type 2 + villous atrophy of progressively more severe degrees, denominated 3a-partial atrophy, 3b-subtotal atrophy, and 3c-total atrophy)

A Mouse Model of CD

To shed light on the reciprocal contribution of the innate and adaptive immune responses in the context of CD pathogen-

esis, a triple transgenic mouse model has been recently developed [101]. It does not only express the human HLA-DQ8 (I), one of the alleles that predisposes to CD development, but reproduces also the overexpression of IL-15 in both the small intestinal epithelium (II) and the lamina propria (II), similarly to what is observed in active CD patients. This animal model spontaneously develops villous atrophy after protracted gluten ingestion, which is restored upon gluten withdrawal. Notably, when lacking one of the three above-mentioned trans-genes, the intestinal damage was prevented, thus showing that overexpression of IL-15 in both mucosal compartments (epithelium and lamina propria) is required for the development of villous atrophy upon gluten exposure in a genetically predisposed host. Importantly, this model allowed to demonstrate that the gluten-specific $CD4^+$ T cells, the HLA-DQ8 expression, the cytokine IFN-γ, and the enzyme tTG have a crucial role in tissue destruction, suggesting new avenues for potential therapeutic strategies. Lastly, the availability of an animal model in which the tissue damage develops and reverts in response to gluten provides a key tool for testing newly developed drugs.

Clinical Presentations

Atrophy of the villi of various degrees (from partial to total) with crypt hyperplasia results from the inflammatory changes described in the previous section and is correlated with the extent of the specific autoantibodies (see below under "Diagnosing Celiac Disease"). It should be noted however that the severity of the villous atrophy does not predict the long-term outcome of CD [102]. Malabsorption of nutrients thus ensues and with this, understandably, a number of gastrointestinal symptoms. But the clinical manifestations of celiac disease go well beyond the intestine, so that basically all systems and organs can be involved, leading to a variety of extra-intestinal manifestations. In fact, CD presentation has substantially changed over time [103–106], both in children and in adults, moving from a malabsorptive disorder causing blatant GI symptoms and malnutrition (with failure to thrive being a common occurrence), to a more subtle condition causing a variety of extra-intestinal manifestations that can occur either with or without a concomitant GI involvement. Moreover, it is also well known that CD may be entirely asymptomatic, as we have come to know from screening unselected populations or first-degree relatives of known patients. It is no surprise therefore that the diagnostic rate around the globe is quite dismal, as both the general public and the health-care providers may miss the oligo- or a-symptomatic individuals as well as those whose symptoms do not include obvious GI involvement [107].

Table 40.1 lists the main presentations of CD. As it can be seen, the clinical manifestations can be protean, thus making the diagnosis not obvious in most cases. When CD has its onset in infancy and very early childhood, the GI manifestations typically prevail and can be quite aggressive, resulting in a clinical picture of malnutrition and failure to thrive, often associated with a protein-losing enteropathy. Subsequently, however, the onset may be more subtle, and extra-intestinal manifestations become more common.

GI Manifestations

Among the most frequent GI manifestations of CD are abdominal pain and bloating [108]. Chronic or intermitted diarrhea, characterized by bulky, foul-smelling, greasy stool, is also a very common symptom in children with CD. Its occurrence, however, is progressively becoming less frequent than in the past [105]. Counterintuitively, long-standing and occasionally severe constipation can be the presenting manifestation in a significant portion of both

Table 40.1 Main presenting signs, symptoms, and laboratory changes in celiac children

GI manifestations	Extra-GI manifestations	Laboratory findings
Abdominal pain, bloating	Weight loss/failure to thrive	Elevated CD-specific antibodies Hepertransaminasemia
Diarrhea	Delayed puberty	Anemia (especially iron-deficient)
Vomiting	Short stature	Vitamin D deficiency
Constipation	Dermatitis herpetiformis	Osteopenia
Anorexia	Aphthous ulcers/geographical tongue	
Intussusception	Dental enamel defects	
Celiac crisis	Osteopenia, osteoporosis	
	Arthritis, arthralgia	
	Alopecia areata	
	Headaches, migraine	
	Peripheral neuropathy	
	Idiopathic seizures	
	Sad mood/depression	
	Psychiatric disorders	
	Fatigue	

pediatric and adult patients [108–110]. Constipation appears to be related to a well-documented delay in oro-cecal transit time [111] possibly caused in part by disturbed upper GI motor function [112]. Other presenting symptoms related to the GI tract are vomiting (especially in infants and toddlers), weight loss, or failure to thrive. Poor appetite, occasionally severe like a true anorexia [113], can be an accompanying symptom, leading – particularly in cases of delayed diagnosis – to severe malnutrition and cachexia (Fig. 40.2). However, it should be noted that children with CD can also be overweight or obese, as well documented in the literature [114–116], so that the absence of malnutrition should by no means rule out the possibility of CD.

Recurrent intussusception, an uncommon but important GI sign, was first described in 1997 [117] and is now a well-described occurrence in CD children, known to be more frequent in undiagnosed CD children than in control populations [118].

More rarely CD can have its onset with a dramatic, acute presentation, referred to as "celiac crisis." This term describes a sudden onset episode of explosive diarrhea associated with protein-losing enteropathy, hypoalbuminemia, and profound electrolyte abnormalities with metabolic acidosis leading to dehydration and lethargy, occurring most commonly before the diagnosis of CD and in young children, although documented in adults too [119].

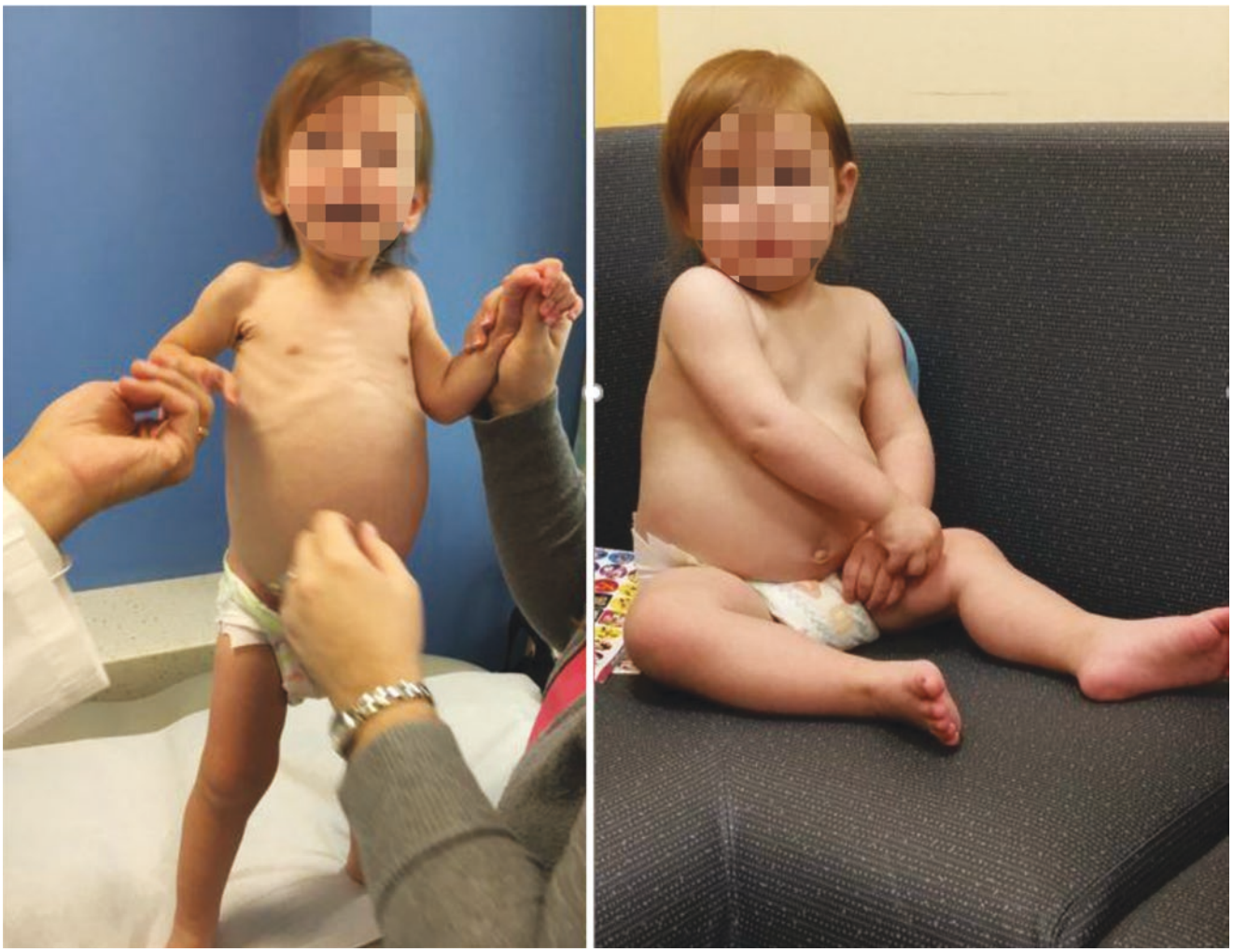

Fig. 40.2 Typical GI presentation in young children. Left panel: a 19-month-old girl at the time of diagnosis. Right panel: the same patient 6 weeks later

Extra-Intestinal Manifestations and Laboratory Changes

Extra-intestinal symptoms (Table 40.1) occur at similar rates in children and in adults [120]. In children, short stature, fatigue, and headache appear to be the most common. Failure of proper height gain can be an isolated initial presentation of CD [120, 121] and can be found in up to 10% of children being investigated for short stature. If diagnosed with enough time before the onset of puberty, it typically responds well to the GFD [120]. While some of the extra-intestinal manifestations such as weight loss, fatigue, short stature, and delayed puberty can be attributed to nutritional deficiencies and their metabolic consequences, others follow different and often poorly understood pathways.

Dermatitis herpetiformis (*DH*) (Fig. 40.3) is considered the skin manifestations typical of celiac disease [122]: autoantibodies against epidermal transglutaminase (TG3), the supposed autoantigen of DH, are at play in this condition also characterized – but by no means constantly – by villous atrophy. Rare in children, DH affects mostly teenagers and adults who present symmetrical, pruritic blisters followed by erosions, excoriations, and hyperpigmentation. The most involved sites are mainly the extensor surfaces of elbows and knees, followed by shoulders, buttocks, sacral region, and face. Itching of variable intensity, scratching, and burning sensation immediately preceding the development of lesions are common. The diagnosis of DH is made by a direct immunofluorescence biopsy of unaffected skin in close proximity to an active lesion [123]. Pathognomonic granular IgA deposits at the dermo-epidermal junction are seen (2 D). Its treatment is based on a strict GFD that is typically extremely effective. Dapsone and/or other drugs can be used during the early phase of treatment, until the GFD becomes effective [124].

Oral manifestations are well described too, among them, dental enamel hypoplasia, geographical tongue, and aphthous ulcers. Dental enamel hypoplasia has been reported with a prevalence ranging from 10% to 97% [125–129],

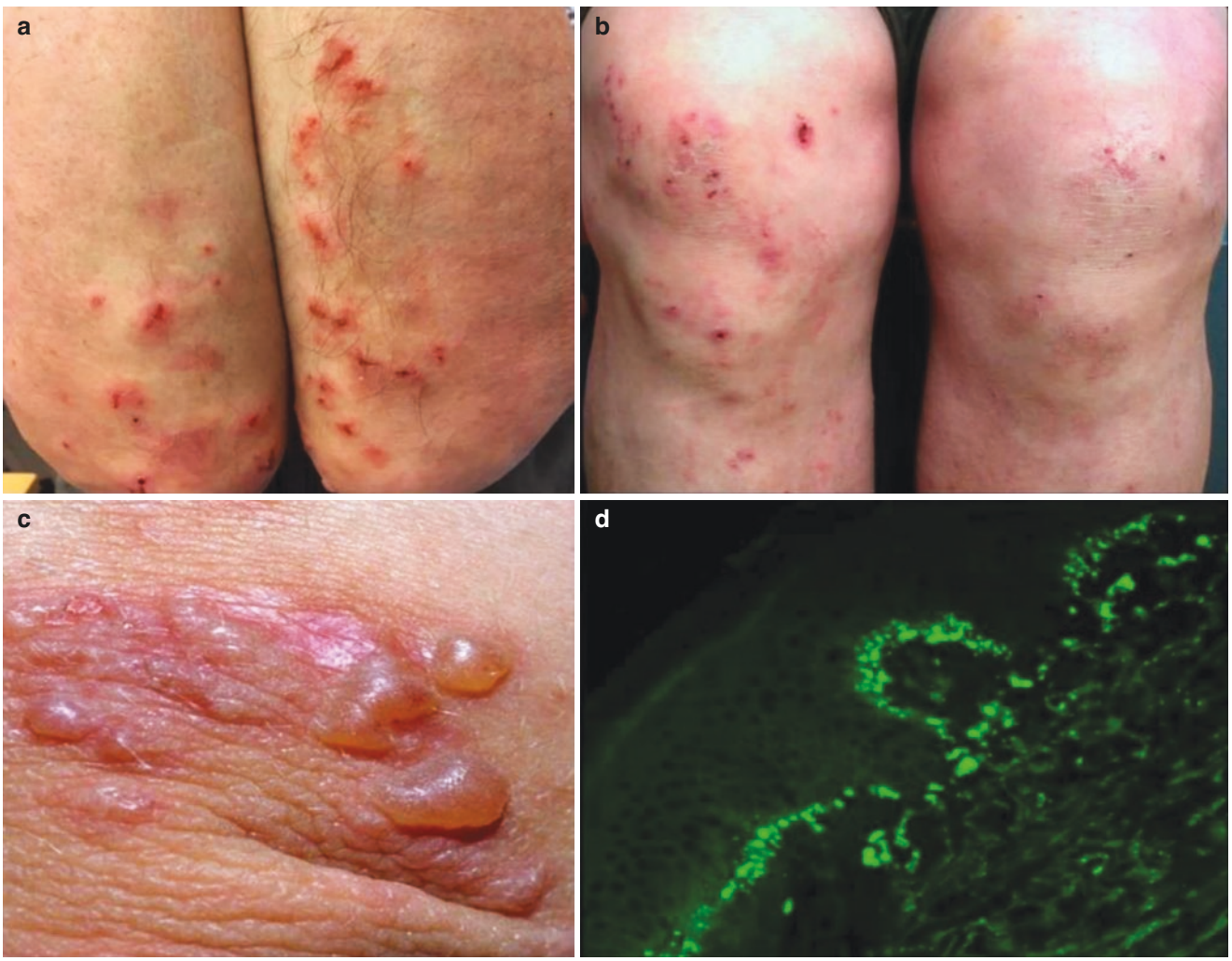

Fig. 40.3 Dermatitis herpetiformis [from Ref. [122], reprinted with permission]. Typical scratched papules and macules on the elbows (**a**) and on the knees (**b**). Fresh small blisters on the elbow (**c**). Direct immunofluorescence showing granular IgA deposits in the basal membrane zone between the epidermis and dermis (**d**)

more commonly in children. This defect is thought to be secondary to nutritional deficiencies and immune disturbances during the period of enamel formation, mostly the first 7 years of life [130]. Other enamel defects that can be associated to CD are enamel pitting, grooving, and partial or complete loss of the enamel. Of note, dental enamel defects can be found in children in the absence of any other symptoms, as documented in a large epidemiological study in Italian children [131], and are therefore a useful screening tool. Aphthous ulcers can be present in children with CD [132], although they are neither characteristic nor specific to CD since they can also be associated with other medical conditions such as inflammatory bowel disease and Behcet's disease. CD has been reported as more prevalent in children with geographic tongue, a benign inflammatory condition of unknown etiology usually involving the dorsal surface and lateral borders of the tongue, than in the general population [133]. As in many of the extra-intestinal manifestations, geographic tongue too can occur in the absence of GI involvement.

Low bone mineral density is found in many newly diagnosed patients [134] and, in some cases, especially in adults, is advanced to osteoporosis and can be associated with fractures [135]. The etiology of such bone alterations in CD is multifactorial, thought to be mostly secondary to the combination of intestinal malabsorption and chronic inflammation. Recently, higher serum OPG, telopeptide, and lower serum pro-peptide have been found in adult CD patients with low bone mineral density, suggesting an increased bone turnover [136]. Low bone mineral density apparently responds well to the GFD in CD children, and recent data suggest that adult patients too can improve their bone mineral density after some years on GFD [137].

Joint involvement, though not too common, has been described in children and adults with CD [138], and it is suggested that patients presenting with unexplained articular manifestations be tested for CD [139, 140].

Alopecia areata is a common form of hair loss in childhood, affecting about 1–2% of the population and characterized by a sudden onset of patchy hair loss on the scalp. It is considered an autoimmune condition, and it has been shown to be more common in children with CD [141], once again even in the complete absence of GI manifestations. Good response to the GFD has been observed [120].

Children with CD have an increased frequency of *neurological symptoms* compared to controls [142]. The most common is definitely headache [143–145] that appears in 18% of children [145] and in most cases responds well to the GFD [120]; but also peripheral neuropathy [142] and seizures [146] are well described; ataxia on the other hand is described almost exclusively in adult cases [147]. The prevalence of epilepsy in CD children also appears to be higher than expected, as reported in a recent large epidemiologic study [148]. Seizures are often generalized tonic-clonic, but partial and occasionally absence seizures are also reported. In some patients with CD and epilepsy refractory to antiepileptic drugs, seizures have been controlled with a GFD [149, 150]. Hence, it is recommended that patients with epilepsy without a clear etiology should be screened for CD, as an early diagnosis and treatment might be beneficial. Relatively common in children and especially in adolescents are also psychiatric issues [151] including anxiety, often with recurrent panic attacks, hallucinations, and depression that appear to persist into adult life [151]. A moderately increased risk of suicide in CD patients has also been reported [152]. Interestingly, there is some evidence that the GFD may help in alleviating depression in celiac adolescents [153].

Elevation of *liver* transaminases is well described in pediatric CD, either idiopathic or associated to autoimmune hepatitis, and is thought to be present in about one-third of all children with CD [154], while CD is found in 12% of children with mild unexplained hypertransaminasemia. Of note, GFD normalizes transaminase levels in most patients with CD within a few months.

Anemia in CD children can be the end result of several different, and sometimes combined, causes [155]; however, the single most common type of anemia is due to iron deficiency (IDA). IDA may be the only clinical abnormality identified in many patients and can be the presenting feature of CD, especially in older children and adults [156]. Anemia at CD diagnosis is associated with more severe histological and serological presentation in children [156, 157]. However, even when asymptomatic, CD can lead to IDA; in a large series of patients with asymptomatic CD, IDA was indeed found in almost half of the patients, with adults having a higher incidence than children: 46% versus 35% [158].

The most common *vitamin deficiency* found in children with CD at diagnosis is vitamin D [159, 160], a factor likely to contribute to the low bone mineral density [161]. Testing for its level is therefore recommended [162] at diagnosis, when instruction on age-appropriate intake of calcium and vitamin D, in order to provide adequate replenishments, should be provided during nutritional counseling along with the need for exercise to promote bone health.

Disease Associations

Autoimmune and some chromosomal disorders have been known for a long time to be associated with CD. In addition, several large-scale epidemiological investigations have revealed a growing number of additional associated conditions, although in most cases the reasons for such associations and their clinical relevance remain unclear (Table 40.2). Among the conditions with increased prevalence in CD, mostly occurring however in adults, are asthma [163],

Table 40.2 Main conditions associated with celiac disease

Diseases	Autoimmune conditions	Genetic/ chromosomal disorders
Juvenile idiopathic arthritis	Type 1 diabetes mellitus	Selective IgA deficiency
Asthma	Thyroid disease: Hashimoto thyroiditis Graves-Basedow disease	Down syndrome
Eosinophilic esophagitis	Addison disease	Turner syndrome
Gastroesophageal reflux	Psoriasis	Williams-Beuren syndrome
Pancreatitis	Systemic lupus	
Adrenal insufficiency		
Idiopathic dilated cardiomyopathy		
Atrial fibrillation		
Cataracts		
Uveitis		
Renal disease		
Stroke		
Chronic obstructive pulmonary disease		

chronic obstructive pulmonary disease [164], cardiac disorders and strokes [165–167], gastroesophageal reflux disease [168], pancreatitis [169], ocular complications [170, 171], idiopathic dilated cardiomyopathy [165], renal disease [172], adrenal insufficiency [173], and systemic lupus [174].

Of note, in children, the association with eosinophilic esophagitis has been described [169] and the effect of GFD in both conditions has been assessed [175].

Autoimmune Conditions

CD shows a strong association with autoimmune disorders, thought to be mostly due to a shared genetic component in the HLA region [2]. The best described association is with T1DM, a condition sharing common pathogenetic mechanisms with CD [176]. Indeed, CD has a prevalence of approximately 10% among T1DM patients [177–179]. About two-thirds of children diagnosed with CD after T1DM onset have elevated levels of celiac antibodies at the time of T1DM diagnosis or within the first 24 months; however, an additional 40% of patients develop CD a few years after T1DM onset [180], and even adults with a long history of T1DM show a progressively higher prevalence of CD [181]. Thus, it is recommended that T1DM children be repeatedly tested for CD. Of note, it has been shown that the presence or absence of GI symptoms in children with T1DM has no predictable value for biopsy-confirmed CD or not [182]. In fact, many diabetic children who come to be diagnosed with CD have minimal or no symptoms. Testing children with T1DM for CD is recommended by the American Diabetes Association; the International Society for Pediatric and Adolescent Diabetes; the British Society of Paediatric Gastroenterology, Hepatology and Nutrition; the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) in their 2012 as well as 2020 guidelines; and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition [1, 183–187]. As patients with T1DM can however have mildly elevated titters of TTG-IgA independently of CD, it is recommended to proceed with the confirmatory biopsy only when their titer is >3 times the upper limit of normal (ULN). Clearly, once diagnosed with CD, children with T1DM need to follow a strict GFD; however, its effects on diabetic control are unclear. In fact, glycemic control, both in children with or without malabsorption, has been found either improved or unaffected [181].

Autoimmune thyroid diseases (both Hashimoto's thyroiditis and Graves-Basedow disease) are more frequent in CD children than in controls [188]. In these patients, GFD does not appear to prevent the progression of the autoimmune process [189].

Addison disease, mostly in adults, has an increased prevalence in CD patients as well [190]. Psoriasis appears to be more prevalent in CD patients, too [191].

The issue of whether the onset of autoimmune disorders in CD patients is favored by the ingestion of gluten (either before or after diagnosis) remains controversial. An increased prevalence of autoimmune disorders was found in parallel with the increasing age at diagnosis of CD [192], suggesting that prolonged exposure to gluten may indeed favor the onset of autoimmune conditions. However, as further studies on this issue have revealed conflicting results, the issue is still unclear [193].

Genetic/Chromosomal Disorders

Selective IgA deficiency has also been associated with CD. In fact, about 14% of IgA-deficient children are celiac [194] and about 2% of CD children are IgA-deficient [195]. While clinically not relevant, this association bears important implications in the strategy for diagnosing CD (see below). CD prevalence has been found to be between five- and tenfold higher in children with Down syndrome [3, 4], Turner syndrome [5], and Williams-Beuren syndrome [6] than in the general population; hence, they need to be screened for CD. Finally, given the high prevalence among first-degree relatives of CD patients [196], they must be screened too for CD even if asymptomatic.

Diagnosing CD

Given the various clinical manifestations of CD, which can also be asymptomatic, as well as the association with other conditions, clearly the first requisite for diagnosis is a high degree of suspicion. Table 40.3 lists the circumstances that demand testing for CD regardless of the presence or absence of symptoms. The availability of very sensitive and specific IgA autoantibodies, generated in the small intestinal mucosa and detectable in the serum, such as the tTG-IgA or anti-endomysium IgA (EMA-IgA) [197], has given the physician a powerful screening tool. In addition, another class of antibodies has also been found valuable in screening and in following up CD patients: the anti-deamidated gliadin peptide (DGP) antibodies (both IgA and IgG), produced against the gliadin peptides after they have been modified by the tTG [198].

The first step in screening for CD in subjects of any age who are on a gluten-containing diet is the serum level of tTG-IgA [1, 185, 186, 199], considered to possess the highest degree of sensitivity, thus basically avoiding missing any case of CD. Total serum IgA levels however need to be added in order to ascertain that the individual can produce tTG-IgA and identify IgA-deficient subjects. In those cases, both tTG-IgG and DGP-IgG [200, 201] can be usefully checked as markers of CD.

In 2012, an ad hoc task force of ESPGHAN published revised criteria [1] and produced an evidence-based algorithm that allowed the physician to skip the duodenal biopsy under certain circumstances, namely, in patients showing a genetic asset and a history compatible with CD and very elevated titers (more than ten times the ULN) of tTG-IgA, along with a positive EMA titer. In the years that followed, many observations were carried out in various parts of the world, including in North America [202], basically validating this suggested approach. Then in 2020, after gathering multicenter experience, ESPGHAN went further eliminating the need to document a consistent genetic asset as well as the presence of CD-compatible symptoms, stating "If TGA-IgA is ≥10 times the ULN and the family agrees, the no-biopsy diagnosis may be applied, provided EMA-IgA will test positive in a second blood sample. *HLA-DQ2/-DQ8 determination and symptoms are not obligatory criteria*. CD diagnosis can be accurately established with or without duodenal biopsies if given recommendations are followed" [186]. It is estimated that by applying this approach, about 50% of children presenting with a suspected CD can be diagnosed without a biopsy. In addition, a recent study in the USA [203] estimated that using this algorithm could avoid "between 4891 and 7738 pediatric endoscopies per year in the United States," thus resulting in a considerable cost saving. The algorithm presented in Fig. 40.4 (from Ref. [186] summarizes accordingly the diagnostic steps for a child/teenager suspected of CD).

Table 40.3 Subjects to be screened for CD

All those presenting the conditions listed in Tables 40.1 and 40.2
First-degree relatives of celiac patients
Autoimmune conditions
Type 1 diabetes
Autoimmune thyroiditis
Autoimmune hepatitis
Addison's disease
Hashimoto Thyroiditis
Graves-Basedow disease
Genetic/chromosomal disorders
Selective IgA deficiency
Down syndrome
Turner syndrome
Williams-Beuren syndrome

Subjects with normal serum IgA and normal tTG-IgA CD can be excluded, given the high sensitivity of the test; however, the rare seronegative CD patient has been described [204], more commonly among adults [205]. Thus, in the presence of a strong clinical suspicion, it would be appropriate to still proceed with the diagnostic biopsy even with normal CD-specific antibodies. A recent consensus statement [206] recommends that – after having ruled out alternative causes for villous atrophy – patients with suspected CD who are seronegative but have villous atrophy and are HLA-DQ2 or DQ8-positive should be put on a GFD and then re-assessed endoscopically after 1–3 years on a GFD, to evaluate resolution of the villous atrophy that would confirm the diagnosis. On another hand, if the subject has positive tTG-IgA, but at titers lower than 10 times normal and/or with a negative EMA, then the EGD would be necessary.

During the upper endoscopy it is important to obtain at least four biopsies from the distal duodenum, and one or two from the bulb; otherwise the diagnostic yield may be jeopardized by the occasional patchy duodenal lesions. In interpreting the pathology of the duodenal biopsies, caution must be exerted for findings of Marsh type 1, especially when not supported by positive serology. In fact, such increase in IELs ("lymphocytic duodenosis") has been found to be due to CD in no more than 16–39% of cases [207, 208]. Thus, additional conditions (Table 40.4) must be carefully ruled out before concluding for CD. As for those with a Marsh type 0, the call is even more delicate. In fact, patients could be defined as "potential CD" if tTG-IgA and EMA titers are positive; while in case of low titers of tTG-IgA and a negative EMA, the possibility of a false-positive tTG-IgA must be considered. This occurrence is especially common, for unclear reasons, in children with T1DM, where tTG-IgA has also been found to spontaneously normalize [209–211]. Asymptomatic patients with mild elevation of tTG-IgA but a normal biopsy should therefore remain on a gluten-containing diet and be carefully monitored.

Potential Celiac Disease

More complex is the decision about the need for a GFD for true "potential" CD children and adolescents. A large prospective study conducted in Italy [212] with up to 12 years of follow-up of 280 of these children investigated the possible risk factors associated with the development of villous atrophy. The children underwent serologic tests twice a year and a small bowel biopsy every 2 years. During the follow-up period, 42 (15%) of 280 children developed villous atrophy, whereas 89 (32%) children no longer tested positive for tTG-IgA or EMA. The cumulative incidence of progression to villous atrophy was 43% at 12 years. Of interest, the factors most strongly associated with development of villous atrophy were numbers of γδ IELs followed by age (younger children having less chances of developing full-blown CD) and HLA-DQ2 homozygosity.

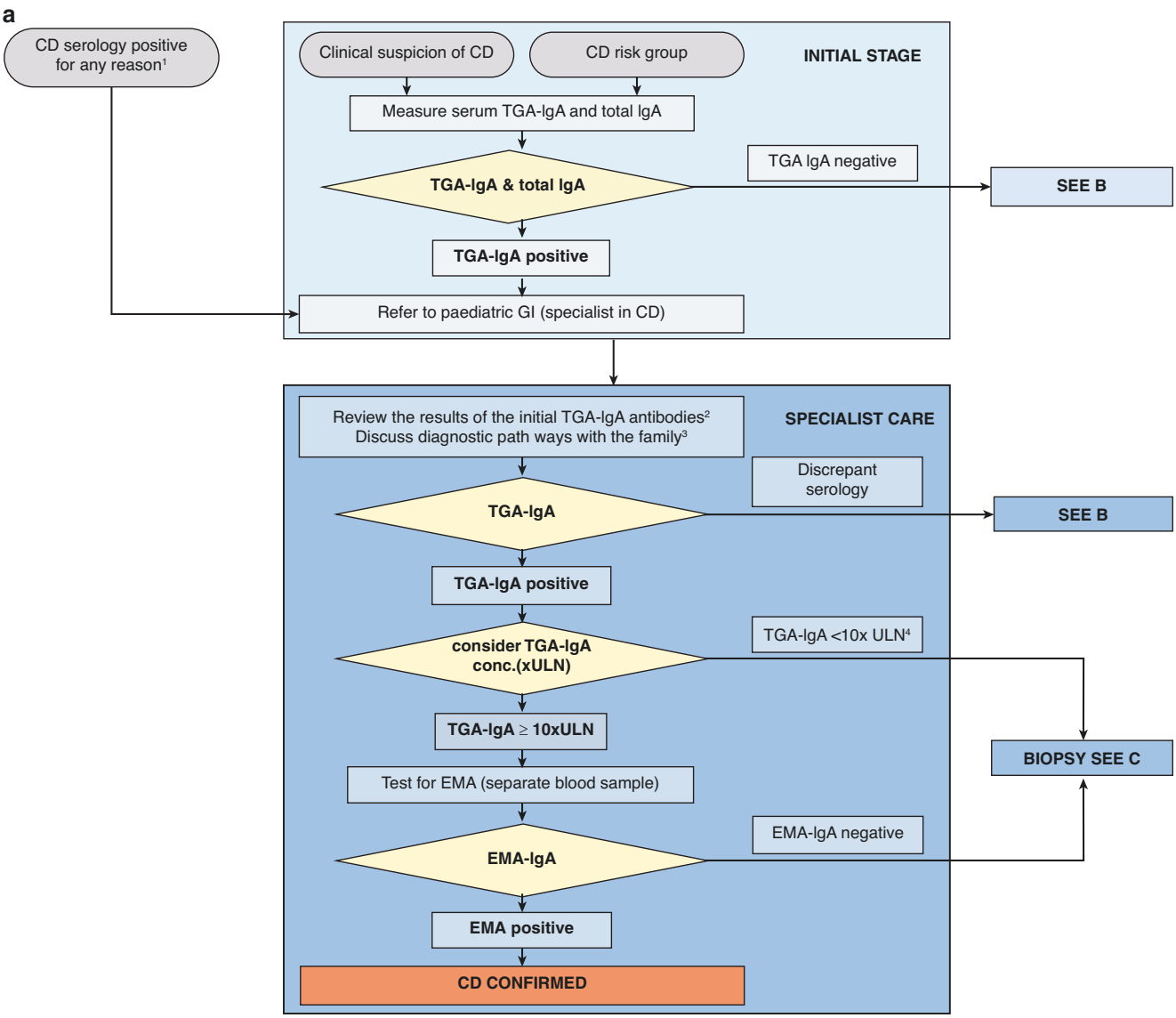

Fig. 40.4 Algorithm for diagnosis of celiac disease [from [186], reprinted with permission]. (**a**) in IgA-competent subjects, (**b**) in IgA-deficient subjects, and (**c**) for biopsy

[1] Other than TGA-IgA, including point-of-care tests and DGP

[2] Check the value also in relation to the cutoff and repeat the test if questionable or borderline. No need to retest if done with validated assay with calibration curve. Test with conventional TGA-IgA test if positive POCT and TGA has not been measured quantitatively

[3] Convey the message that the diagnosis of celiac disease with or without biopsy confirms the need for a lifelong gluten-free diet and that re-evaluation after introduction of the diet would need prolonged re-exposure to gluten with a series of further investigations

[4] If TGA-IgA is only borderline positive, confirm sufficient gluten intake and consider re-testing of TGA-IgA and EMA

[5] Low for age or <0.2 g/L above the age of 3 years

[6] For example, dermatitis herpetiformis, in which serology is frequently negative

[7] The cutoff for normal numbers of IEL is >25 cells/100 enterocytes. The action done at the initial stage in primary care (light blue) is separated from specialist care (dark blue)

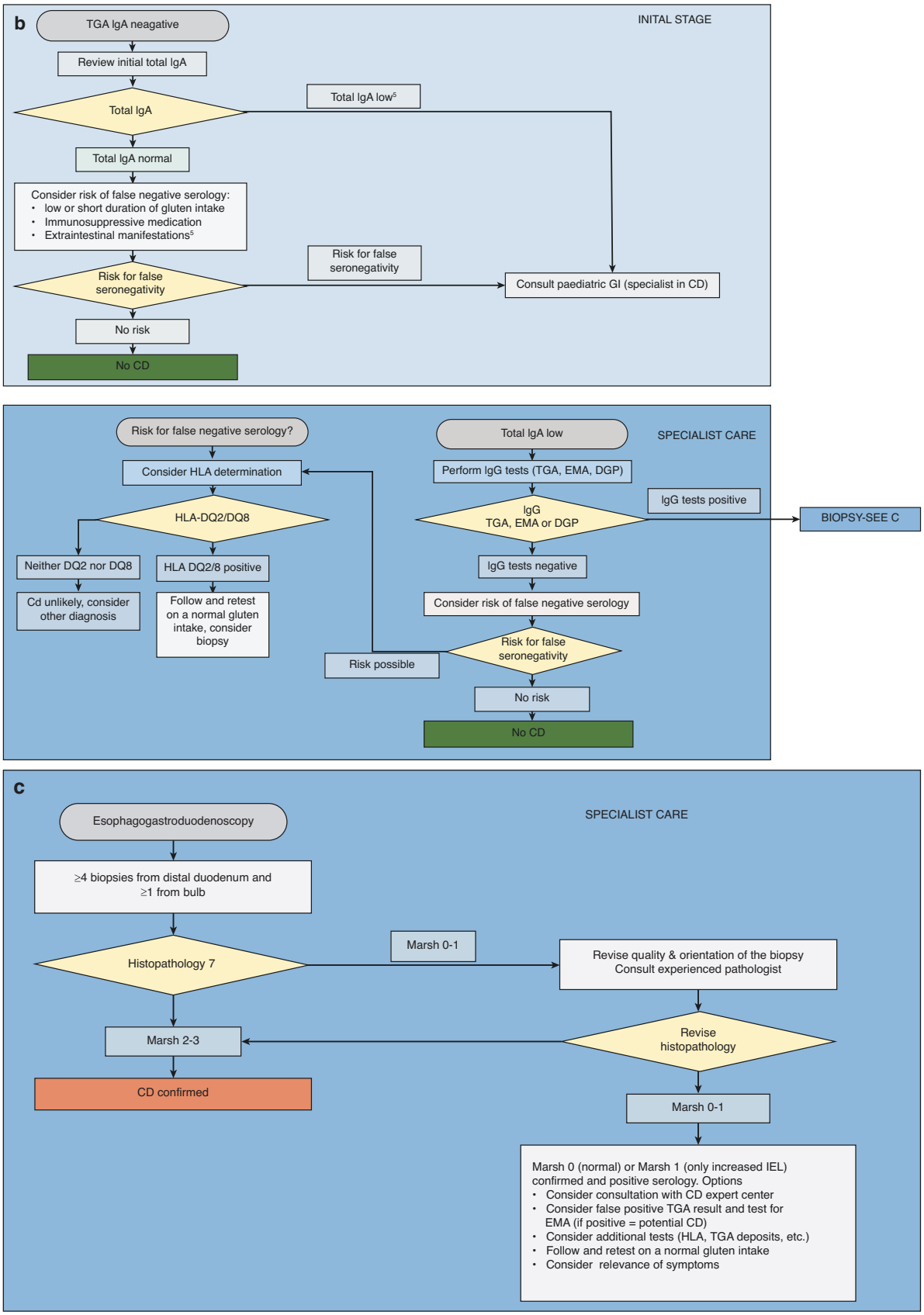

Fig. 40.4 (continued)

Table 40.4 Conditions causing lymphocytic duodenosis (Marsh type 1 changes)

Celiac disease
H. pylori gastritis
Small-bowel bacterial overgrowth
NSAID and other drugs
Immune dysregulation
Crohn's disease
Food protein-induced enteropathy
Infections
NSAID (nonsteroidal anti-inflammatory drugs)

Thus, in practical terms the decision on whether to put a potential CD patient on a GFD must be taken with great care, on an individual basis, after having properly informed the parents and obtained their full agreement. It goes without saying that if a potential celiac is left on gluten, he/she must be periodically followed.

Nonresponsive Celiac Disease

While the GFD appears to be very effective in children, a minute portion of patients would have persistent elevated serology and/or symptoms, configuring a condition of "nonresponsive CD." In these circumstances, a careful analysis of all potential causes is needed, keeping in mind that by far the commonest is ongoing ingestion of gluten that can be detected and resolved by the use of a rigorously supervised and strict GFD [213]. Among the less frequent causes of nonresponsive CD, recently a superimposed cow's milk protein allergic enteropathy has also been described [214]. The term "refractory CD" (RCD) instead refers to the persistence of intestinal villous atrophy (with or without persistent positive serology) for more than 1–2 years despite a strict GFD in patients with CD. This condition occurs rarely and its diagnosis remains difficult. RCD has been subdivided into two subgroups according to the normal (RCDI) or abnormal (RCDII) IELs phenotype, the latter considered as a low-grade intraepithelial lymphoma with a poor prognosis [215]. Despite some debate, there is scarce evidence for the existence of this condition in children. Hence, before concluding that a child presents true RCD, it is strongly recommended investigating properly other causes. As mentioned, in addition to ongoing inadvertent ingestion of gluten, they would include conditions such as Crohn's disease, autoimmune enteropathy, small bowel bacterial overgrowth, cow's milk protein allergy, and pancreatic insufficiency.

Treatment

CD is a lifelong condition and a strict GFD is currently the only available effective treatment. The dietary restriction must include wheat, rye, barley, and their derivatives such as triticale, spelt, and kamut. Other cereals such as rice, corn, and buckwheat are perfectly safe for CD patients and can be used as wheat substitutes.

How strict should the diet be? While the exact amount of gluten that can be tolerated daily by CD patients is unknown and is likely to be subject to a wide interindividual variability, there is evidence that a daily intake of less than 10 mg is safe for all patients, while the majority of them would react at levels equal or higher than 100 mg per day [216, 217]. This very strict diet proves to be very hard to follow, and in fact there are numerous reports in the literature showing that many celiac patients, especially in the adult population, experience an involuntary intake of gluten exceeding the safe amounts [218, 219]. A source of concern for risk of contamination with gluten in children is their school activities. A recent test on surfaces that could be contaminated with gluten revealed that there is indeed a potential for gluten exposure at school that varied among different materials (higher for paper mache and baking products) [220]. Inadequate adherence to the GFD has been recently assessed in a systematic review gathering almost 8000 children [221] that showed rates of adherence ranging from 23% to 98%. As expected, adolescents were more at risk of nonadherence, while children whose parents had good knowledge about CD adhered more strictly. Nonadherence is associated with patient growth, symptoms, and quality of life. To explain at least in part such lack of adherence, one must note that children (especially older ones and teenagers) may not present any symptom after inadvertent gluten ingestion or sporadic intentional consumption of gluten-containing products. On the other end of the spectrum, there is some evidence that using "hypervigilance" in adhering to the diet may be detrimental, especially in teenagers, on quality of life [222]. Additionally, the GFD often results in choice of manufactured GF products that are higher in sugar and fats and lacking in fiber and micronutrients, so that children on GFD experience unbalanced nutrition, from excessive caloric intake [223] to micronutrient deficiency [223–225]. Furthermore, in a study on a large number of adults, it was found that those following a GFD had significantly higher urine levels of total arsenic and blood levels of mercury, lead, and cadmium, possibly as a result of larger intake of fish and rice [226]. Recently, assessing the dietary preferences of a cohort of children with CD, we find that processed food

items have become a staple of the GFD, and in many cases, these products were consumed to the exclusion of healthy alternatives [227]. Variable effects on body mass index (BMI) have been observed after beginning the GFD, likely the result of different approaches to feeding habits in various sociocultural environments [228–230].

Safety of consuming oats (a non-gluten-containing cereal) had been a matter of debate for a very long time, with reiterated proofs – summarized in a recent meta-analysis [231] that non-contaminated oats are indeed perfectly safe; in addition, a good source of fiber and nutrients, including oats, adds variety and palatability to the diet.

The GFD, particularly in pediatric age, has a strong record of efficacy in resolving the presenting symptoms of CD [232]: while GI symptoms appear to be better responding than extra-intestinal ones to GFD, constipation appears to have the worst rate of improvement for both children and adults at only 74% and 58%, respectively [233]. Among extra-intestinal manifestations short stature and psychiatric disorders showed the worst rates of improvement in children [233]. GFD resulted in greater rates of improvements for GI than for extra-GI manifestations, both children and adults [120, 234]. Furthermore, in general, improvement in children was more rapid than in adults. Even more important, healing of the damaged duodenal mucosa has been documented in the vast majority of children after 1–2 years on GFD [235, 236]. Thus, even if up to 20% of the patients *re-biopsied in large part due to persistent symptoms* (hence, a small minority of the total) may have persistent mucosal damage [237], systemic re-biopsying in CD children on GFD is not recommended [238].

Assessing dietary compliance is not always an easy task, since serology often fails to reveal slight transgressions. Therefore, a periodical follow-up is needed, and the importance of a lifelong diet should be constantly reinforced, especially to asymptomatic patients. Recently, a test has been developed [239] and later marketed that allows the detection in samples of stools (more sensitive) and urine (less sensitive) of even minimal amounts of gluten that had been ingested in the previous 48 h. By using this method, studies in adults found that gluten ingestion occurs frequently despite efforts to follow a strict GFD [240, 241]. While this is a useful tool in verifying if recently developed symptoms can be attributed to a suspected, inadvertent ingestion of gluten, it must be kept in mind that it cannot be relied upon to verify prior, long-standing compliance.

In summary, following a strict GFD is not easy, may expose to nutritional deficiencies/excesses and risks, and carries an impact on quality of life, especially in adults. In addition, there is a subgroup of CD patients (i.e., RCD) who fail to respond even to a strict GFD, thus reinforcing the concept that GFD could not be considered a cure for all CD patients. As a result, in the last decade research has focused on numerous alternative forms of treatment.

Each one of these recently reviewed [242] alternatives is aimed at attacking a specific point in the pathogenesis of CD (Fig. 40.5): from genetically modifying the gliadin-containing grains in order to eliminate the toxic peptides, to enzymatically degrading the ingested gluten (endopeptidases) in the stomach before they reach the small intestine (latiglutenase [243] and AN-PEP [244]); from utilizing probiotics to affect the microbiota in symptomatic patients on GFD [245] to polymers that would sequester gliadin peptides (BL-7010) preventing them from crossing the intestinal barrier [246]; from agents like larazotide able to modulate the tight junctions in order to block the entry of such peptides [247] to inhibiting the deamidation of gliadin peptides by the TG2 (ZED 1227); and from blocking these peptides from adhering to the HLA-DQ2/HLA-DQ8 receptors [248] to interfering at various levels with the subsequent steps of immune activation by CD4 T-cells and their cytokines. Among the latter, recently the development of a promising therapeutic vaccine (Nexvax2) has been halted as it "did not provide statistically meaningful protection from gluten exposure for celiac disease patients when compared with placebo" [249]. All of the other approaches listed above are in various phases of active development [242], from preclinical to phase II clinical trials, but at this time none has been FDA-approved.

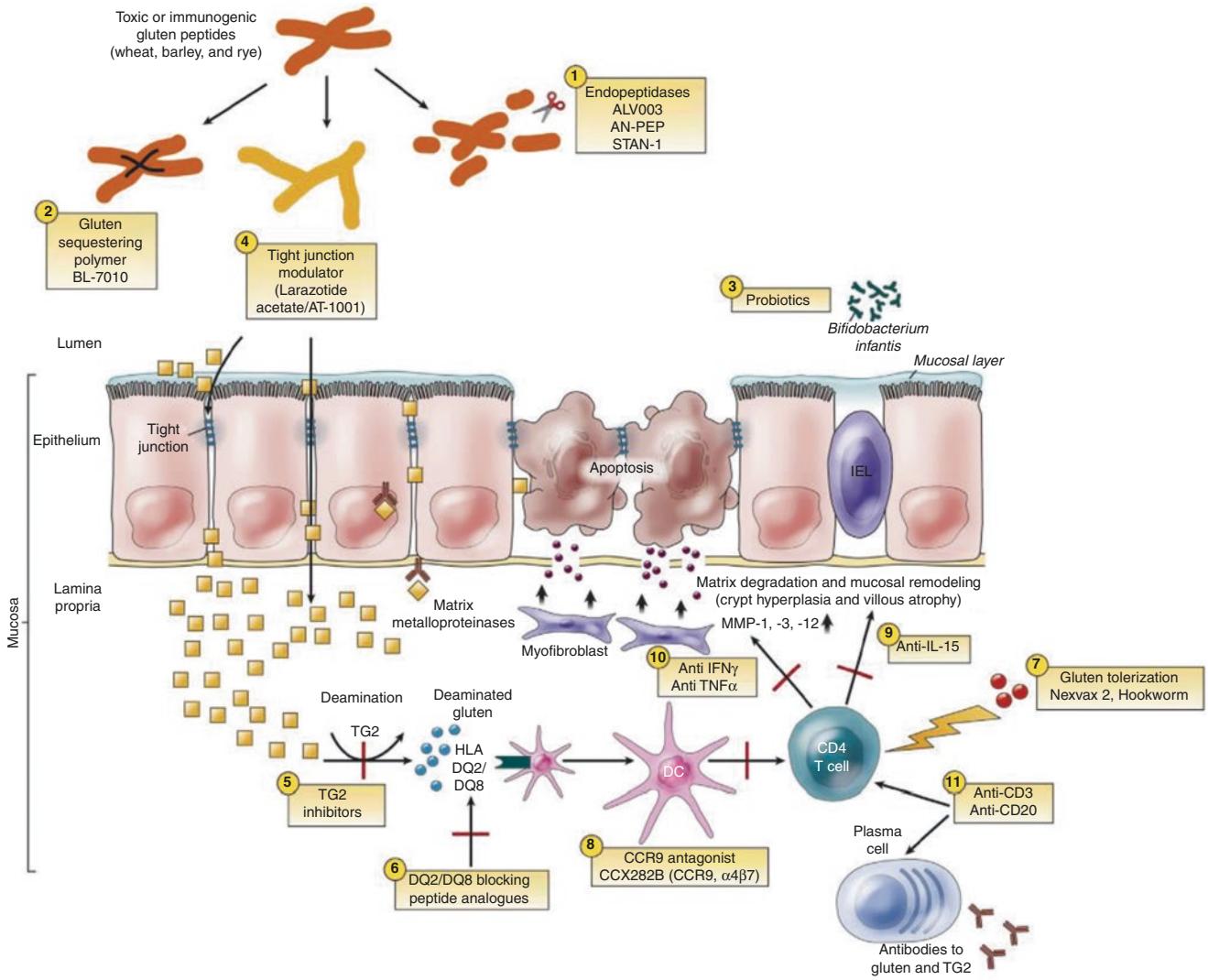

Fig. 40.5 New nondietary therapies for celiac disease (from Ref. [242] with permission). (1) Endopeptidases: Latiglutenase (formerly ALV003), AN-PEP, and STAN-1 degrade gluten into nonimmunogenic particles, thereby alleviating mucosal injury. (2) Gluten-sequestering polymer: BL-7010 binds to intraluminal gliadin and prevents its release and breakdown into immunogenic peptides. (3) Probiotics: Bifidobacterium infantis protects epithelial cells from damage caused by gliadin by downregulating the proinflammatory immune response. (4) Tight junction modulator: Larazotide acetate/AT-1001 works as a tight junction modulator to prevent gliadin-induced epithelial permeability. (5) TG2 inhibitor: Blocks the transformation of native gliadin peptides to the far more antigenically potent deamidated gliadin peptides. (6) DQ2/DQ8 blocking peptide analogues prevent presentation of gliadin from activating T cells. (7) Gluten tolerization: Nexvax2 and hookworm (*Necator americanus*) inoculation aim to downregulate the immune response to gluten. (8) CCR9 antagonist: CCX282B blocks this chemokine receptor to block lymphocyte homing. (9) Anti-IL-15 is a monoclonal antibody that may prevent immune-mediated tissue destruction. (10) Anti IFN-γ may prevent inflammation. (11) Anti-CD3 antibodies suppress gluten-activated T cells; anti-CD20 antibodies suppress B cells

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Cystic Fibrosis

41

Zev Davidovics and Michael Wilschanski

Introduction

This chapter focuses on disease pathobiology of the gastrointestinal, nutritional, and hepatic manifestations of cystic fibrosis (CF).

CF is an autosomal recessive multisystem disease, the highest incidence being in individuals of North European descent. Intense follow-up and treatment have improved the median survival to nearly 50 years, and in many countries, over 50% of individuals are older than 18 years of age. Even though the main cause of death is chronic lung disease, gastroenterological symptoms feature prominently as 85% of patients have exocrine pancreatic insufficiency (PI), and around 10% suffer from significant liver disease.

Cloned in 1989, the CF transmembrane conductance regulator gene is situated on chromosome 7. The CFTR protein has two nucleotide-binding domains, two membrane-spanning domains, and a unique regulatory domain. This protein acts as a cyclic adenosine monophosphate (cAMP)-dependent chloride channel and localizes to the apical membrane of secretory and absorptive epithelial cells within the intestine, liver, sweat gland, pancreas, airway, and the vas deferens [1].

An inability of duct lumens to hydrate macromolecules is responsible for a number of CF manifestations. A body of pathologic evidence indicates that organ damage can be traced to ductal or glandular plugging by macromolecules that have precipitated in concentrated secretions. For instance, mucus secretions in the bronchi and the intestine are viscid and inspissated, and the crypts are distended as though obstructed. A deficit of ductular fluid flow and altered biochemical and physiologic properties of secretions underlie this situation.

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The CFTR Gene

There are over 2000 mutations in the CFTR gene, and they have been divided into six classes [2]. This classification system provides the opportunity to evaluate the effects of genotype on phenotype by considering the effects of CFTR mutations on CFTR protein function. Nevertheless, it should be emphasized that this classification system is limited by the fact that the functional consequences of many rare mutations (particularly missense mutations) are unknown and cannot be predicted.

Class I mutations include mostly nonsense, frameshift, or missense mutations that result in defective protein biosynthesis (truncation, deletion, etc.). The nonfunctional products are efficiently degraded within the cell. Class II mutations, such as F508del, produce a misfolded functional CFTR protein, which is degraded intracellularly preventing trafficking to the apical surface of the cell. Class III mutations affect channel activation by preventing binding and hydrolysis of ATP at one of the two nucleotide-binding domains (NBD1, NBD2). Class IV mutations produce a protein with impaired function due to abnormal anion conduction. Due to a variety of mechanisms including abnormal splicing, promoter mutations, or inefficient trafficking, class V mutations result in a reduced number of normally functioning CFTR molecules on the apical surface. Class VI mutations result from truncation of the C-terminus of CFTR and produce a functional protein which is unstable at the apical membrane surface. Mutations belonging to classes I–III and VI confer little or no functional CFTR at the apical membrane. As a consequence, inheritance of homozygous or compound heterozygous mutations belonging to these classes is predicted to have severe consequences. Mutations belonging to classes IV and V on the other hand which confer (or are presumed to confer) some residual CFTR-mediated channel function would be expected to have milder consequences. In fact, it is patients with at least one mutation

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belonging to classes IV or V who generally present with symptoms in late childhood or adulthood.

Diagnosis of CF

The diagnosis is clear if the patient has a sweat chloride concentration >60 mmol/L with at least one recognized phenotypic characteristic which includes chronic sinopulmonary disease, salt loss syndromes, and, in males, obstructive azoospermia [3, 4]. The majority of CF patients will have one established CF causing mutation on each CFTR allele. The diagnosis of CF should not be automatically considered if the sweat chloride concentration is >60 mmol/L. There is a list of disorders with high sweat tests which is expanding (Table 41.1).

At the other end of the spectrum, problems arise when there is a CF phenotype in one or more organ systems, and the sweat chloride level is borderline (30–60 mmol/L).

Electrophysiological testing using nasal potential difference (NPD) measurements may aid in the diagnosis of CF patients with normal or borderline sweat tests and negative or uninformative genetic test results [5, 6]. This test measures transepithelial sodium and chloride transport in the nasal epithelium. The function of CFTR in chloride secretion is intimately related to the inwardly directed sodium transport via the epithelial sodium channel (ENaC). The activity of these two channels is the basis for NPD. A catheter is placed under the inferior turbinate and is connected via a series of electrodes to a voltmeter and recorder. The nasal epithelium is perfused with solutions which inhibit sodium transport and activate chloride transport. In CF patients, the readings are markedly dissimilar from controls. Another electrophysiological tool is the intestinal current measurement (ICM) which measures CFTR function ex vivo in rectal biopsy using a modified Ussing chamber [7]. Testing should only be carried out by an experienced individual in a specialist research center where objective reference values have been established.

Table 41.1 Conditions other than CF associated with raised sweat electrolyte concentration

Glucose-6-phosphatase
Adrenal insufficiency
Familial hypoparathyroidism
Nephrogenic diabetes insipidus
Mauriac's syndrome
Familial cholestatic syndrome
Anorexia nervosa
Severe malnutrition
Atopic dermatitis
Keratitis–ichthyosis–deafness (KID) syndrome
Fucosidosis
Pseudohypoaldosteronism
Patients undergoing prostaglandin infusions
Hyperchlorhidrosis caused by homozygous mutation in CA12, encoding carbonic anhydrase XII
Accurate references lacking
Glucose-6-phosphate-1-dehydrogenase deficiency
Ectodermal dysplasia
Hypothyroidism

CFTR Dysfunction: Gastrointestinal Consequences

Exocrine Pancreatic Abnormalities

Exocrine Pancreatic Function

In pancreatic ductal epithelia, the CFTR protein is highly expressed, allowing fluids and anion to enter the ductal lumen. Thereby, luminal chloride is exchanged for bicarbonate, giving evidence that CFTR is permeable to bicarbonate [8]. According to Quinton's hypothesis, the defect in bicarbonate transport is indeed the primary defect in CF [9]. This results in an increased volume of alkaline fluid, allowing the acinar cells to remain in a soluble state, due to the highly concentrated proteins secreted. Absent or reduced CFTR channel function impairs chloride and bicarbonate to enter the ducts which results in reduced volume of a more acidic fluid [10]. The acidic milieu within the acinar lumen also leads to impaired reuptake of GP2, the zymogen-granule-associated protein [11]. The consequences of mutations in the CFTR gene have been demonstrated by pancreatic function studies showing that CF patients have low flow secretions with a high protein concentration, presumably which will precipitate in the duct lumina causing obstruction, damage, and atrophy (Fig. 41.1).

These changes begin in utero, and after birth, the process continues with the small duct obstruction leading to a larger duct obstruction. For several months afterward, there is a release of proteins, originating in the pancreas, into the bloodstream. This pathological process forms the basis for the immunoreactive trypsinogen (IRT), the neonatal screening test for CF. The reason has yet to be determined, but, interestingly enough, the infant is asymptomatic while this wholesale destruction of the exocrine pancreas is occurring. Eventually, this process results in severe inflammatory changes, obstruction of ducts by mucus and calcium-containing debris, the destruction of acini, and generalized fibrosis. The high IRT does show that some exocrine pancreatic tissue is still present and may have a bearing on possible small molecule therapy targeted at the remainder of the pancreas which may rescue enough tissue to cause viability of the remaining pancreas. This of course contradicts the popular belief that the pancreas is entirely nonfunctioning at birth (see below CFTR correctors and potentiators).

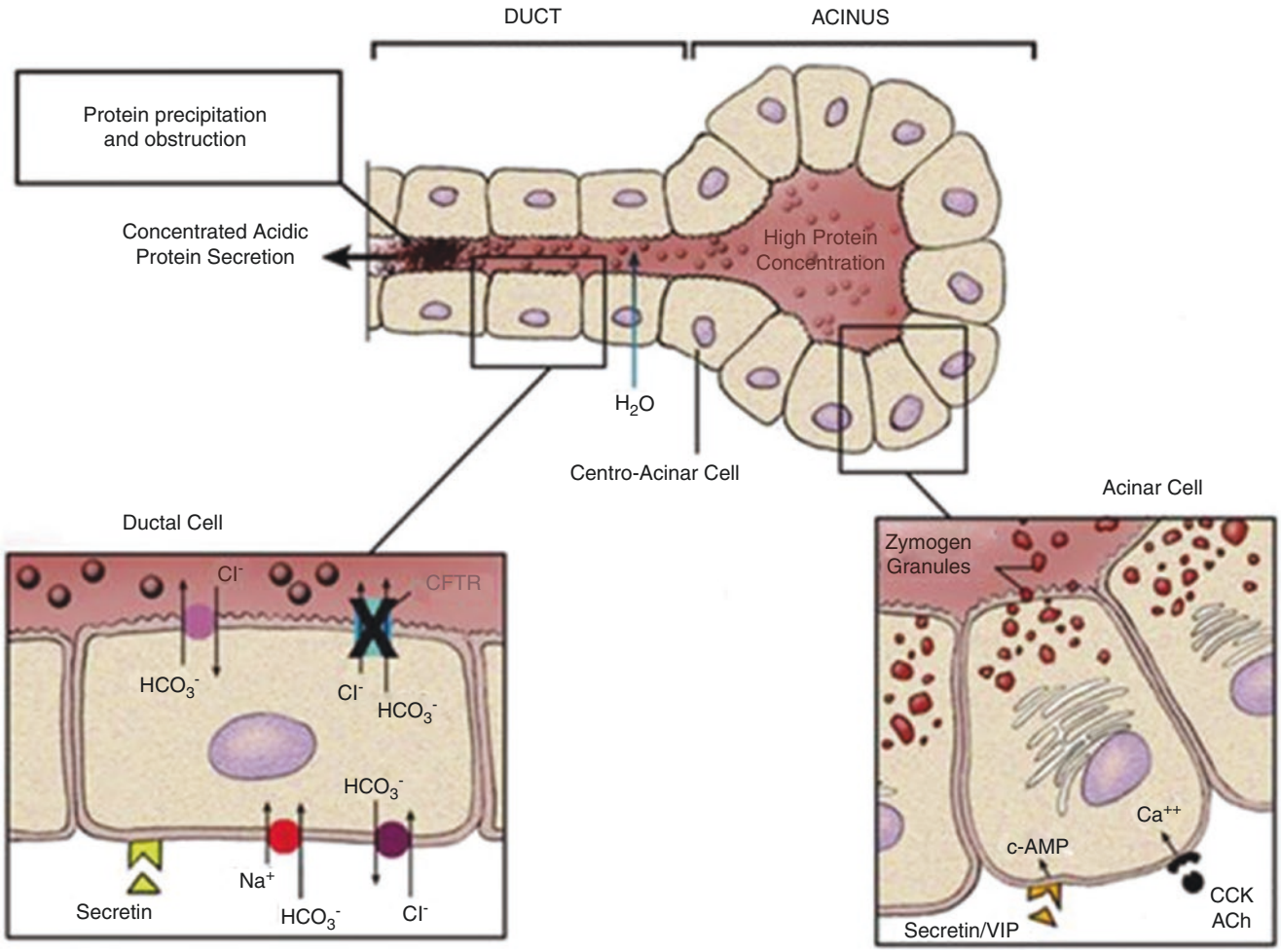

Fig. 41.1 Pathogenesis of pancreatic disease in cystic fibrosis (CF) [12]. Acinar cells secrete large quantities of protein, primarily in the form of digestive enzymes, into the acinar lumen. Under normal circumstances, anions (chloride and bicarbonate) are secreted into the ductal lumen via the cystic fibrosis transmembrane regulator (*CFTR*) and bicarbonate exchangers. This provides a driving force for the movement of fluid into the lumen of the duct and maintains the solubility of secreted proteins in a dilute, alkaline solution. In CF, impaired anion transport into the proximal ducts results in decreased secretion of more acidic fluid, which leads to precipitation of secreted proteins. Intraluminal obstruction of the ducts then causes progressive pancreatic damage and atrophy (Reproduced from Ref. [12], Copyright 2007, with permission from BMJ Publishing Group Ltd). *cAMP* cyclic adenosine monophosphate, *ACh* acetylcholine, *CCK* cholecystokinin, *VIP* vasoactive intestinal peptide

One of the most remarkable observations is that genetic factors exquisitely influence the degree of pancreatic disease and its rate of progression. CF patients are classified as pancreatic insufficient (PI) or pancreatic sufficient (PS). PI patients comprise approximately 85% of all CF patients. PI patients present with maldigestion, as evidenced by steatorrhea (fecal fat greater than 7% of fat intake in infants over 6 months of age and over 15% under 6 months of age), requiring these patients to have pancreatic enzyme replacement therapy (PERT) with meals. PS patients, however, have evidence of pancreatic damage, but retain sufficient endogenous exocrine pancreatic function to sustain normal digestion [13] due to the fact that ductal CFTR in PS patients is partially functional, thereby allowing anions and fluid to enter the ductal lumen. This provides further evidence that mutant CFTR may be acting on other apical anion exchangers rather than on chloride ion conduction itself [14].

The exocrine pancreatic status is directly linked to genotype [15]. Analysis of particular CFTR mutations in patients with pancreatic phenotypes (PI vs. PS) revealed two categories of alleles: "severe" and "mild." Patients homozygous or compound heterozygous for severe alleles belonging to classes I, II, III, or VI confer PI as opposed to patients with a mild class IV or V allele even when the second mutation is severe. These patients are termed "PS." Plausible explanation of this observation finds all known mild alleles belong to class IV or class V, all of which are (or predicted to be) associated with some residual chloride channel activity at the epithelial apical membranes. Although these mutations confer sufficient CFTR function to prevent the pancreas to be

completely destroyed, many PS patients have reduced exocrine pancreatic capacity and are associated with an increased risk of pancreatitis, as discussed below. A very small number (2–3%) of patients carrying severe mutations on both alleles are PS at diagnosis; however, most patients will experience a gradual transition from PS to PI. A few missense mutations (e.g., G85E) confer a variable pancreatic phenotype.

Recurrent Pancreatitis

Many PS patients have reduced exocrine pancreatic capacity and are associated with an increased risk of pancreatitis, even though mild mutations confer sufficient CFTR function to prevent the pancreas to be completely destroyed. As first reported by Shwachman in 1975, recurrent acute and chronic pancreatitis are relatively infrequent complications of CF. In this retrospective study, only 0.5% of CF patients had pancreatitis. Durno et al. reported more recently an incidence of 1.7% in a cohort of over 1000 patients followed over a period of 30 years [16]. All of the pancreatitis patients were classified as PS, but this subgroup of PS patients, in fact, appeared to be highly susceptible to pancreatitis, since almost one in five was affected by this complication. In a seminal paper of the largest study to date of CF PS patients, Ooi et al. determined the association between severity of CFTR genotype and the risk of pancreatitis [17]. They examined a large cohort of 277 PS patients from 2 CF centers, of which 62 had well-documented pancreatitis. The mutations were divided into three main groups: severe, moderate–severe, and mild in using a novel pancreatic insufficiency prevalence (PIP) score. It was found that the proportion of patients who developed pancreatitis was significantly greater for genotypes in the mild group than the moderate–severe group. Thus, the more mild mutations were associated with increased risk of pancreatitis. CFTR mutations may contribute to the development of pancreatitis along with other genetic and environmental factors [18].

Diagnosis of Pancreatic Phenotype

With the increasing worldwide use of neonatal screening programs, patients may present with symptoms of CF or be entirely asymptomatic. Making a determination in all patients, whether the patient is PI or PS is essential to enable a rational use of oral PERT [19]. When patients have steatorrhea (oil droplets on stool microscopy), hypoalbuminemia, and low fat-soluble vitamin levels, the diagnosis of PI is straightforward. However, the lack of these findings does not exclude PI; therefore, more formal testing is required. In the past, direct exocrine pancreatic stimulation testing was administered. Unfortunately, this was an invasive, time-consuming procedure and not widely used in most centers. Indirect pancreatic testing with 72-h fecal fat measurements should be encouraged and be used as follow-up for pancreatic exocrine status and response to therapy. Due to the technical difficulties surrounding the performance of this test, most laboratories are utilizing the fecal elastase-1 test instead, with its main advantage being that it does not need prolonged stool collection. Pancreatic elastase is secreted into the duodenum and is found at relatively high concentrations in stool (>500 μg/gm stool).

The use of fecal pancreatic elastase-1 has now become a common diagnostic test for assessing exocrine pancreatic status. The advantages and limitations of fecal elastase-1 in CF have been discussed by Kalnins et al. in a review article [20]. The cutoff levels of fecal elastase for PI range between 100 and 200 μg/g stool; a majority of centers use the upper level of 200 μg/g stool. With the use of 200 μg/g stool weight, some patients may be falsely identified as PI. The Toronto group compared stool elastase values to the 72-h fecal fat in both known PI and sufficient patients and found that an elastase value of 100 μg/g stool had a 99% predictive value in ruling out PI based on an abnormal fecal fat. Cade et al. showed that patients with pancreatic sufficiency on the basis of a normal fecal fat balance study were found to have fecal elastase values in the range of 100–200 μg/g stool; elastase levels were compared with the fecal fat test coefficient of fat absorption (CFA) [21]. Whether defining PI as <93% or 90% CFA, cutoff levels of fecal elastase of < 100 μg or <200 μg/g stool for either monoclonal or polyclonal methods were positive predictors of insufficient pancreatic function. However, patients with pancreatic sufficiency were not included in this study population. Moreover, these observations question the validity of defining the cutoff for PI as a fecal elastase value below 200 μg/g stool. In a different study of 21 known PS patients, a poor correlation was found between fecal fat excretion and elastase-1 [22]. The fecal elastase result will define clearly whether a patient is PI or sufficient, in a majority of cases, provided the test is done accurately. In situations such as in acute diarrhea, short gut, or stool from an ileostomy, where stool is more watery, fecal elastase levels may be lower than expected; therefore, it would be advisable to wait until the diarrhea resolves, or until a sample that is more formed is available. A definitive answer on pancreatic status may not be provided in those patients with less common genetic variations, where there are no supportive clinical features of PI, and fecal elastase values are often borderline. In such cases, elastase can be used to monitor pancreatic status, in conjunction with ongoing evaluation of clinical and nutritional status [23].

Oral Pancreatic Enzyme Replacement Therapy

Prior to 2010, pancreatic enzyme products were exempt from the Federal Food, Drug, and Cosmetic Act of 1938 and did not require approval of the Food and Drug Administration (FDA). Since then, a plethora of products became available to the public without the need for strict preclinical and clinical studies. The FDA has since mandated that all manufacturers of pancreatic enzyme products in the USA must seek approval.

Currently, there are various preparations approved and available for use [24–30]. During one study on Creon, a fixed dose of 72,000 lipase units with meals and 36,000 with snacks was evaluated. The study was a double-blind, randomized, placebo-controlled trial of 54 adult patients with chronic pancreatitis or post-pancreatic surgery. The treatment arm showed a difference in CFA of 19.3% over placebo (85.6 vs. 66.3).

Most commonly available PERT products showed a similar nutrient absorption rate between 83% and 87% fat absorption. The dosages of these products are based on the lipase units contained in the product. It is now common for patients to change from one product to another using a 1:1 lipase ratio and then titrating for maximum efficacy. The North American CF Foundation has published guidelines according to the age of the patient and according to the grams of fat ingested per day [31].

The importance of the correct enzyme ingestion in infants and children is a major concern. There is often difficulty in feeding infants capsules or microspheres however small they may be. The continued use of the unprotected powder enzymes for infants until 1 year of age is common in some centers. In infants, these enzymes may have some advantage over the enteric-coated versions in certain situations. The efficacy of unprotected powder enzymes (in tablet or powder form) has not been directly compared to the enteric-coated version in infants, but infants treated with this approach do achieve growth and weight gain, proving their efficacy [32]. In addition to their use for infants, unprotected powder enzymes are often used to help digest enteral tube feedings where oral administration of enzymes is not possible, or when jejunostomy feeds are required.

Breastfeeding mothers should be instructed on proper infant mouth care after enzyme delivery when the unprotected powder enzymes are provided to these infants. It is recommended that soft cotton swabs or a washcloth dipped in sterile water be used to wipe the inside of the infant's mouth and inside the gums. This will prove to be sufficient to prevent gum erosion to the infant and nipple irritation to the mother.

For infants with meconium ileus requiring surgery or those with an ileostomy, the powder enzymes may provide the advantage of immediate release in the duodenum. Theoretically, this will improve nutrient digestion compared to the pH-sensitive, delayed release enteric-coated microsphere enzyme products. Tablets without a protective coating can be crushed if the powder version in capsules or in bottles is not readily available.

The production of specially designed enzymes for the small child has been a recent advancement. A multicenter cross-over study in CF infants who were randomized to receive CfC (Creon for children) or regular enzyme for 2-week periods was performed; it compared a spoon administration containing 5000 lipase units with the standard Creon 10,000 capsule [33]. The parental preference was the primary end point; over 75% of the parents preferred CfC over the standard preparation.

Theoretically, the addition of bicarbonate to enteric-coated enzyme preparations might raise the proximal intestinal pH and thereby optimize dissolution of the enteric coating and improve enzyme activity. There were conflicting studies on efficacy when compared to standard, pH-sensitive microsphere enzymes. One study demonstrated that there was no improvement when compared to a standard enzyme preparation [34]; another study showed improvement in fat absorption with the bicarbonate-containing enzyme [35]. However, in both studies, approximately 80% fat absorption was achieved using the bicarbonate-containing enzyme, and Kalnins et al. [34] found the same degree of fat absorption with the conventional enteric-coated enzyme product.

Oral PERT is not formulated for use with continuous enteral tube feedings especially overnight. Parents often add standard formulation enzymes several times a night and this causes hardship. A new therapy that advances EN tube feeding is a single-use digestive enzyme cartridge connected in line with the EN feeding set. Lipase is bound to a beaded polymer retained in the cartridge. The efficacy of this device has been confirmed in two studies with improvement in gastrointestinal symptoms and laboratory improvement of omega-3 fatty acid levels in erythrocytes [36, 37].

An important factor for clinicians and families of a CF patient or those with CF to understand and appreciate is that although enzyme therapy for those with PI allows for normal growth and weight gain in most individuals with CF, unfortunately, it does not completely correct nutrient malabsorption. There are several reasons for incomplete nutrient digestion with the currently available enzyme products. A proportion of the unprotected powder enzymes or tablets may become inactivated by prolonged exposure to gastric acid. This results in decreased duodenal active enzyme recovery. Enteric-coated microspheres, which dissolve at a pH of >5.5, may only be released in the ileum if duodenal

milieu does not reach this pH as occurs in CF. From prior intubation studies, evidence is confirmed that release of enzymes from enteric-coated microspheres is delayed in CF, and thus, they are delivered beyond the duodenum, even as far distal as the ileum. This results in a nutrient digestion occurring in the more distal small intestine, but not in the duodenum and proximal jejunum as in health. Not only maldigestion but also malabsorption contributes to the insufficient assimilation of nutrients. Fatty acid absorption as well as the digestion of triglycerides is impaired in subjects with CF [38]. Other contributing factors to nutrient malabsorption include incomplete lipid solubilization caused by a depleted bile salt pool and thick intestinal mucus. This may affect the unstirred water layer, reducing absorption of fatty acids into the small intestine epithelium. Therefore, a degree of malabsorption is to be expected for reasons mentioned. Consequently, achieving >90% nutrient digestion as evaluated by 72-h fecal fat studies is not likely to occur in a majority of patients with CF.

Patients should not be automatically encouraged to increase their PERT intake if they experience gastrointestinal symptoms such as abdominal pain, bloating, or loose stools, as there are many other etiologies for these symptoms including compliance. For persistent symptoms, a fat balance study should be performed to titrate dose and proton pump inhibitor (PPI) should be considered. After which, if the patient does not experience any improvement, investigations for non-pancreatic disease should be explored. Awareness of these factors by both clinicians and patients will help to guide a rational approach to enzyme therapy in CF; an individualized approach to treatment is recommended.

Hepatobiliary Disease

There are a wide variety of hepatobiliary disorders associated with CF (Table 41.2); almost all patients with CF have evidence of hepatobiliary disease. There are no clinical consequences for the vast majority of patients.

Table 41.2 Hepatobiliary complications of CF

Hepatic	Approximate frequency (%)
Neonatal cholestasis	5
Steatosis	20–60
Focal biliary cirrhosis	11–70
Multilobular cirrhosis	5–7
Liver failure	Rare
Biliary	
Microgallbladder	5–20
Distended gallbladder	3–20
Cholelithiasis and sludge	10–25
Intrahepatic sludge/stones	Unknown
Extrinsic compression of common bile duct	Unknown
Cholangiocarcinoma	Unknown

The pathognomonic hepatic feature of CF is focal biliary cirrhosis. Intrahepatic biliary ductal secretion is dependent on CFTR-mediated chloride transport for adequate hydration of the lumen (Fig. 41.2). Loss of CFTR function causes the biliary ductules to become obstructed with thick periodic-acid–Schiff positive material leading to acute and chronic periductal inflammation, bile duct proliferation, and increased fibrosis in scattered portal tracts. Remarkably, adjoining portal tracts are frequently normal. Over 40 years ago, postmortem studies showed evidence of mild focal disease in 11% of infants, 27% of those dying at 1 year, and in more than 70% of adults. Clinically, significant portal hypertension resulting from severe multilobular cirrhosis develops in only 5–7% of patients. This is termed "CF-associated liver disease" (CFLD). CFLD is the single most important non-pulmonary cause of death, responsible for 2.5% of overall mortality in CF. The average age of diagnosis of CFLD is 10–11 years of age, and 90% are diagnosed before the age of 20 [39]. Following routine examination or by laboratory evidence of hypersplenism, most patients are asymptomatic and are often identified by the evidence of hepatosplenomegaly. Hepatocellular function remains well preserved for many years, even decades. Splenomegaly is a consistent finding, and the liver edge is often nodular and hard.

About 40–50% of CF patients exhibit intermittent elevations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), or gamma glutamyltransferase (GGT), which are generally 1–2.5 times above the upper reference limits. Biochemical markers of liver disease do not reliably identify patients with multilobular cirrhosis nor do they predict the development of end-stage liver disease. Furthermore, patients with advanced multilobular cirrhosis may have normal liver biochemistry test results. As hyperbilirubinemia is rare, elevated serum gamma globulin level is more likely to be associated with chronic pulmonary inflammation. CFLD occurs predominantly in PI patients. Thus, severe class I, II, or III mutations on both alleles appear to be a risk factor [40]. A male preponderance of severe CFLD has been shown in most studies.

Why a minority of patients with the same severe CFTR mutations progress to CFLD is unclear. However, it has been hypothesized that non-CF modifier genes, such as polymorphisms in genes that upregulate inflammation, fibrosis, or oxidative stress, confer an increased susceptibility. In a multinational gene modifier study of the different candidate genes, the SERPINA 1 Z allele of alpha-1 antitrypsin deficiency was found to be strongly associated with CFLD. This result is intriguing as the CF patient requires a double hit to develop CFLD. Hitherto undefined environmental factors are also likely to be involved.

Gallstones develop in 1–10% of patients with CF. There is also an increased incidence of a variety of intrahepatic and extrahepatic abnormalities of the biliary tree. Nonfunctioning microgallbladders are common, although it

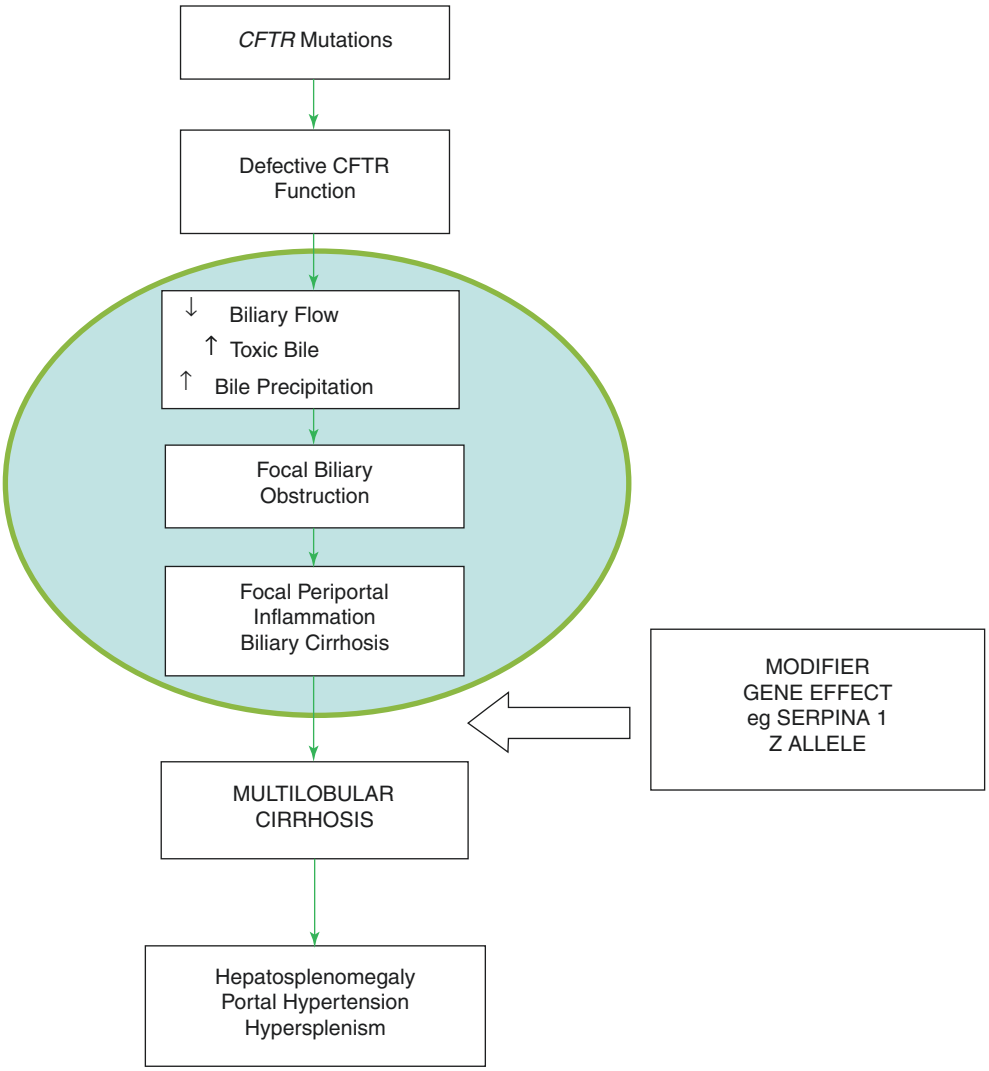

Fig. 41.2 Pathogenesis of cystic fibrosis-related liver disease [12]. Lack of functional CFTR in the biliary ductules causes dehydration, increased concentration, and lack of alkalinization of the duct contents. This causes precipitation of biliary secretions and ductular obstruction. In the majority of patients, the pathologic changes are focal and appear to be clinically inconsequential (*circled area*). However, approximately 5–10% of CF patients proceed to develop clinically significant liver disease with evidence of multilobular cirrhosis and portal hypertension. Susceptibility to more severe liver disease may be modulated by other genetic and/or environmental factors (Reproduced from Ref. [12], Copyright 2007, with permission from BMJ Publishing Group Ltd)

is seen less frequently that patients with CF have distended gallbladders appearing obstructed. Stones may also be commonly visualized within the larger intrahepatic and extrahepatic biliary tree. Debray et al. have proposed using the knockout mouse model that the gallbladder itself, rather than the bile duct epithelia, may be the major site for a bile acid shunt, the cholecystohepatic shunt, from the gallbladder back to the liver [41].

The principal cause of CF-associated liver disease remains controversial; it has been postulated that common duct obstruction results from extrinsic compression by fibrosis within the head of the pancreas. Following ERCP or transhepatic cholangiographic imaging, changes resembling primary sclerosing cholangitis (i.e., beading and stricturing of the intrahepatic and extrahepatic ducts) are quite commonly observed [42]. These changes are likely due to accumulation of protein, mucus, or sludge within the biliary lumina.

An alternative hypothesis of the etiology of CFLD, derived from the histopathological study of liver biopsies, suggests vascular changes, namely, obliteration of portal vein branches with fibrosis leading to portal hypertension [43].

A combined European and North American initiative (ESPGHAN and North American Society for Pediatric Gastroenterology, Hepatology and Nutrition) has convened to reach a uniform definition and classification of CFLD,

which will likely shape diagnostic criteria. In current clinical practice, the diagnosis of CFLD frequently is based on the Debray et al. [44] criteria. A drawback of using these criteria is that varying phenotypical types of liver involvement are included, and it is not always clear whether these are clinically relevant or play a role in development of cirrhosis or portal hypertension. Advances in understanding CFLD pathophysiology will likely guide the discovery of more phenotype specific markers and treatments. It seems reasonable to assume that longitudinal rather than single measurements may become important for the diagnosis and follow-up of CFLD, as well as combinations of diagnostic tests rather than an isolated test or modality.

Diagnosis and Management

Studies are being performed to evaluate early liver disease in CF. Clinical examination is necessary to look for signs of chronic liver disease and organomegaly; routine biochemistry is generally not helpful. The evidence of liver disease is often subclinical until CFLD develops as previously stated. As raised serum liver enzymes in a CF patient may be due to the waxing and waning of liver tests in CF. Persistent increases in liver tests may be due to other diseases entirely; it is imperative to consider hepatitis (infectious or autoimmune), Wilson disease, and other causes of steatosis (malnutrition, diabetes mellitus) as possible causes when reviewing these patients. A small number of infants may present with neonatal cholestasis, in particular those with MI, usually resolving with no long-term effect [45].

In most centers, ultrasonography of the hepatobiliary system is available, and it includes Doppler measurements of flow in the portal vein; however, the positive predictive value of a normal scan and sensitivity is low [46]. Elastography (Fibroscan R), a new test of liver stiffness (which may be a marker of fibrosis), is used in other chronic liver diseases such as hepatitis C. Early diagnosis of CFLD cannot presently be made on the basis of ultrasound alone [47].

Guidelines by Debray et al. [44] recommend that in order to delay progression of CFLD, ursodeoxycholic acid treatment should be initiated. In a Swedish study, there was evidence that biochemistry is improved as is liver histology [48]; however, this treatment and in particular the recommended dose have been challenged after a trial of high-dose ursodeoxycholic acid in another cholestatic liver disease, primary sclerosing cholangitis, was terminated due to severe side effects [49]; further studies are obviously needed.

A number of investigational avenues targeting different aspects of liver disease are being explored. Bile acid analogues: NorUDCA (a side chain-shortened homologue of UDCA with one less methylene group) undergoes cholehepatic shunting leading to a bicarbonate-rich hyperchloresis. This drug has direct anti-inflammatory, anti-fibrotic, and anti-proliferative properties and stimulates alternative bile acid detoxification and elimination routes. It has shown encouraging effects in the clinic improving serum liver tests in primary sclerosing cholangitis, an immune-mediated liver disease with biliary morphological similarities to CFLD, although antibiotics, but not norUDCA, reduced biliary injury in a CF mouse model [50, 51].

Follow-up and management of CFLD are the same as for other chronic liver diseases except for the use of beta-blockade prophylaxis for variceal bleeding. This needs to be thoroughly examined in CF patients in consultation with the pulmonologists. Selection criteria for liver transplantation have not been established. Apart from the routine indications, there appears to be evidence that earlier liver transplantation, before the development of significant and irreversible nutritional and pulmonary deterioration, may be helpful. However, large, long-term studies have been difficult to perform [52].

Intestinal Complications

Meconium Ileus (MI)

Most patients born with MI present within 24–48 h with evidence of intestinal obstruction with abdominal distension, bilious vomiting, and failure to pass meconium. MI occurs in 15–20% of CF patients [53].

Diagnostic aids include a family history of CF and a plain radiograph which may show large distended loops of small bowel and a ground-glass appearance in the right lower quadrant due to inspissated meconium in the ileocecal area.

A diagnostic procedure which may be therapeutic is the performance of a contrast enema. The hypertonic enema may lavage the plug of meconium, and, provided the infant is stable, this procedure may be repeated several times in order to avoid surgery. Surgery is, of course, necessary for complicated MI.

A small proportion presents with perforation in utero with meconium peritonitis and subsequent intra-abdominal calcification which may only be diagnosed incidentally during third trimester ultrasound examinations. Postnatally, up to 50% of MI patients may have additional problems including malrotation with volvulus or intestinal atresia, termed “complicated MI.”

In a consortium which examined over 3700 cases of MI, several apical transporter genes were found to modify meconium ileus [54], thus appearing that modifier gene(s) together with two severe CFTR alleles confers an increased likelihood of MI. It remains possible that modifier genes with or without environmental factors may explain the variability and severity of other intestinal complications such as distal

intestinal obstruction syndrome (DIOS). In a subsequent study, linkage analysis identified a modifier locus for MI on human chromosome 12p13.3 [55].

Distal Intestinal Obstruction Syndrome

Frequently in older children and adults with CF, DIOS, a chronic, recurrent form of partial intestinal obstruction, occurs. DIOS is frequently confused with other common causes of abdominal pain in patients with CF and almost exclusively in those with PI. Unique to CF, DIOS results from a buildup of adherent, thick intestinal contents in the terminal ileum and proximal colon. The reported frequency is variable, consequently, but a recent study shows a prevalence of 18% in adults [56]. DIOS may be related to the specific inflammatory status of the intestine in CF. Intestinal inflammation has been shown in the mouse model [57], and in a study using capsule endoscopy in CF subjects, macroscopic small bowel inflammatory signs were observed and we coined the term "CF enteropathy" [58]. This finding has since been confirmed by other groups [59]. Patients with DIOS usually complain of intermittent episodes of pain, which may or may not be localized to the right lower quadrant. Non-tender, or mildly tender, palpable mass can be felt in the right lower quadrant (although it may not always be localized there). Some patients suffer from intractable chronic pain that is difficult to treat, and on rare occasions, there is complete bowel obstruction.

The European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Cystic Fibrosis and Pancreatic Disease Working Group defines complete DIOS as the combination of (1) complete intestinal obstruction, as evidenced by bilious vomiting and fluid levels on abdominal radiography with (2) a fecal mass in the ileocecum and (3) abdominal pain and or distension. Incomplete or impending DIOS is defined as (1) a short history of abdominal pain and/or distension and (2) a fecal mass in ileocecum but without signs of complete obstruction [60].

While the exact cause of DIOS is unknown, various precipitating factors have been proposed including abnormal properties of intestinal mucus, dehydration of intraluminal contents, slow intestinal transit, poor compliance with pancreatic enzyme therapy, and a prior history of MI and possibly inflammatory changes in the intestine. DIOS should not be confused with simple constipation which is common in CF patients with PI. Although unlike DIOS a right lower quadrant mass is usually not palpable, the rectum will characteristically be full of stool on physical examination, and stooling patterns and consistency are abnormal. Patients undergoing major surgery, such as lung or liver transplant, carry an increased risk of DIOS during the immediate postoperative period.

With other CF-associated or non-CF gastrointestinal disease including appendiceal disease, intussusception, Crohn's disease, fibrosing colonopathy or malignancy, and nonspecific symptoms and signs may occur; therefore, abdominal ultrasound and CT scans may be necessary.

In complete DIOS, the patient usually requires hospitalization and IV rehydration. Patients with incomplete DIOS respond to oral rehydration combined with mineral oil or polyethylene glycol preparations. Enemas of sodium meglumine diatrizoate (Gastrografin or Telebrix) may be performed by experienced radiologists, but the enema must enter the ileocecal valve to get above the obstruction. Treatment of DIOS is still largely empirical due to few randomized controlled trials. Local instillation of diatrizoate in the cecum via colonoscopy has been described [61]. Surgery is seldom required if early aggressive medical management is performed. Laparoscopy and washout are recommended for consideration before resection. A single episode of DIOS is a risk factor for recurrence; maintenance therapy should be considered along with regular follow-up by the gastroenterological service [62].

In a prospective study performed in 10 countries, 112 episodes were prospectively studied. Delayed arrival at hospital and prior weight loss had a significant impact on the time needed for DIOS resolution [63].

Appendiceal Disease

The incidence of acute appendicitis in CF is reported to be lower than in the general population. Nevertheless, patients who develop this complication carry a greater risk of a delayed diagnosis and an increased incidence of complications such as appendiceal abscess. The possible factors include luminal obstruction of the appendix with thick mucus, delayed intervention due to mistaken diagnosis of DIOS, or masking of acute symptoms by chronic antibiotic use.

Intussusception

In 1–2% of adult CF patients, intussusception occurs, approximately 10–20 times higher than in the general population. Most cases are ileo-colic, and 25% are associated with small bowel obstruction [64]. Sticky, muco-feculent material which adheres to the intestinal epithelium may act as the lead point in intussusception. Plain abdominal X-rays are often nonspecific, showing fecal loading, or less often a small bowel obstruction. Presentation is usually with intermittent abdominal pain. Classical appearances of intussusception may be seen on barium studies and include a lobulated soft tissue mass and a "coiled spring" usually situ-

ated in the right iliac fossa. The "doughnut sign" on transverse scan and "pseudo-kidney" on longitudinal scan are some of the ultrasound examination findings. Intussusception is often intermittent and resolves spontaneously, and it may also be observed in asymptomatic patients as an incidental finding during abdominal imaging [65].

Gastroesophageal Reflux Disease

Gastroesophageal reflux disease (GERD) is a multifactorial problem with contributions from both the severity and treatment of lung disease, physiotherapy, transient inappropriate lower esophageal sphincter relaxation, and delayed gastric emptying. GERD is common in CF patients, and there is some evidence that GERD may contribute to the progression of respiratory disease either by pulmonary aspiration of refluxed gastric contents or by neurally mediated reflex bronchoconstriction secondary to irritation of the esophageal mucosa [66]. This is debatable as the GERD may be secondary to the lung disease. Other complications include malnutrition due to increased losses through emesis or reduced energy intake from dysphagia and esophageal strictures. Since silent GERD is common in CF patients, a lack of reported symptoms does not exclude this diagnosis. Bile acids are found in the sputum of children with CF suggesting bile reflux [67]. While esophageal pH or impedance monitoring is helpful, upper endoscopy and biopsy are recommended especially when drug trials have failed in order to define the extent of disease and to rule out other esophageal pathology such as eosinophilic esophagitis.

Fibrosing Colonopathy

A complication of CF involving mainly the colon was reported in young patients with CF in 1994 [68]. The term "fibrosing colonopathy" was used to describe what became recognized as an iatrogenic complication, resulting in considerable morbidity and some mortality. Many patients were initially diagnosed as having inflammatory bowel disease as their symptoms include worsening abdominal pain, intermittent bowel obstruction, and passage of blood and mucus. The proximal colon developed a concentric ring of fibrosis below the submucosa, but in some patients, the concentric fibrosis extended to the entire colon, producing the shrunken "pipe stem" effect. There is considerable hypertrophy of the muscularis mucosa, and the submucosa shows variable degrees of inflammation with eosinophils and mixed inflammatory cells. In the UK and the USA, case-control studies showed a strong statistical association between this complication and dose of pancreatic enzymes (often in excess of 50,000 U lipase/kg body weight) [69]. This led to a reevaluation of the safety of pancreatic enzymes [31]. Notwithstanding, there was no evidence that the excessive doses of enzymes that were used by many clinicians actually improved efficacy or improved abdominal symptoms attributable to maldigestion. Several countries have implemented strict dosing guidelines for PERT. However, a few additional cases of fibrosing colonopathy have been reported in the USA and the UK giving further evidence that "enzyme overdosing" still occurs and also emphasizes the need for close monitoring of enzyme doses in all patients with CF.

Intestinal Infections

The vast majority of patients are asymptomatic, but patients with CF generally demonstrate significantly higher rates of *Clostridium difficile* (32–50%) than the general population (2%), as is the case with healthy neonates. However, on rare occasions severe, potentially fatal cases of pseudomembranous colitis have been reported [70]. Presenting with acute toxic megacolon without diarrhea, the clinical manifestations may be atypical, and absence of diarrhea could be due to the lack of intestinal CFTR function. An immunological protection from diarrhea may be due to chronic antibiotic therapy allowing chronic colonization with this pathogen. Alternatively, the unique intestinal environment in CF patients may provide a favorable milieu for this organism. Another hypothesis is due to an abnormality in receptor binding of the toxin, and there is a reduced risk of clinical complications in CF.

Up to 25% of patients in the general population will have recurrence of their *Clostridium difficile* infection following completion of a treatment course [71]. For recurrent or treatment refractory cases of *Clostridium difficile* infection in the general population, fecal microbiota transplantation (FMT) has proven to be >90% effective in treating the infectious diarrhea [72]. Asymptomatic carriage rates for *Clostridium difficile* are higher for patients with CF than the general population [73], with some studies showing up to 50% of CF patients receiving antibiotics to have *Clostridium difficile*-positive stool [74]. The increased carriage rate along with a decreased infectious rate makes the diagnosis, treatment, and follow-up of infectious *Clostridium difficile* much more difficult in this population. While no studies have specifically looked at the efficacy of FMT for recurrent *Clostridium difficile* infection in patients with CF, one case report by Dunwoody et al. showed that prophylactic FMT in a CF patient with recurrent *Clostridium difficile*-positive diarrhea following antibiotic treatments helped prevent future episodes of diarrhea [75].

CF patients have been reported also to have an increased colonization rate of other bacterial species. This could include *Lactobacillus* spp., *Pseudomonas* spp., *Staphylococcus*, and *Enterococcus*. It has been suggested that CFTR itself acts as a receptor for certain organisms, thereby creating a loss of CFTR affording a form of protection from the consequences of infection. The clinical significance of this is unclear.

In patients with increased diarrhea, abdominal distension, and anorexia, *Giardia lamblia* should be considered as it is a common intestinal parasite and may be more common in patients with PI from any cause including CF [76].

Small-Intestinal Bacterial Overgrowth

CF patients often complain of abdominal pain, bloating, and changes in bowel habit and these common symptoms may be related to the changed microbiota. Indirect evidence points to an estimated prevalence of around 50% of CF patients presenting with small-intestinal bacterial overgrowth (SIBO) [77, 78]. Risk factors include abnormal accumulation of surface mucus (which may allow proliferation and adhesion of bacteria), the reduced alkaline flushing of the upper intestine, and the altered intestinal mucins (which may have limited protective functions). Intestinal dysbiosis is well-documented in cystic fibrosis [79]. Additionally, there is an increased risk of SIBO following intestinal resection due to MI. SIBO may contribute to malabsorption in some patients with CF, although data are limited. The prominent histological feature of the CF mouse's small intestine is mucus accumulation, which occludes the crypts and coats the villus surfaces. The mucus obstruction of the crypts is believed to interfere with innate defense mechanisms of the Paneth cells residing at the base of the crypts, secreting a variety of antibacterial products. One may speculate that SIBO of the CF mouse small intestine is similar to that reported in humans, as there is evidence of intestinal inflammation characterized by increased mucosal infiltration of mast cells and neutrophils and a 40-fold increase in luminal bacteria, and treatment of the bacterial overgrowth reduces mucus accumulation [80].

Emerging evidence suggests that changes in microbiota occur within the first year of life and are associated with early growth failure in the infant with CF [81] and poor clinical outcomes [82]. As stated earlier, intestinal inflammatory changes have been identified macroscopically in people with CF, and this may be due to the altered composition of the gut bacterial ecosystem [58, 59]. Intestinal inflammation as measured by fecal calprotectin is inversely correlated with growth in children with CF [83]. Using a germ-free CF mouse model, Meeker et al. have shown that mutated CFTR alone alters the microbiome [84].

Rectal Prolapse

A feature of CF is rectal prolapse, mainly occurring only in PI patients; however, there have been PS patients observed with prolapsed rectums. Children presenting with recurrent rectal prolapse should be referred for a sweat chloride test to exclude CF.

Celiac Disease and Crohn's Disease in CF

CF was first described as a distinct clinical entity from celiac disease approximately 70 years ago. Reports since then suggest that the prevalence of celiac disease in patients with CF is higher than in the general population [85, 86].

The prevalence rate of Crohn's disease in CF patients is seven times higher than in those reported controls by Lloyd Still et al. [87]. Although there have been many cases reported prior to the description of fibrosing colonopathy, some of them may have been due to the complications of high-dose enzyme therapy and should be considered in CF patients presenting with suspicious symptoms and signs.

Gastrointestinal Malignancy

Early detection of CF especially neonatal screening, improvements in nutrition, bronchial clearance, antibiotics, and organ transplantation have led to a tremendous increase in life expectancy. Currently the median life expectancy in North America for a CF patient born in 2015 is 45 years, and CF now has a majority of patients who are adults. Gastroenterologists are increasingly involved in the care of the multiple complications in CF patients [12]. One of the corollaries of CF becoming an adult disease is that patients suffer from adult problems including in particular gastrointestinal malignancies especially colon cancer [88].

The effect of nonfunctioning CFTR protein on the intestine is being recognized. The impaired electrolyte transport leads to mucosal barrier dysfunction leading to bacterial colonization, and mucosal inflammation. This has been well documented in cftr-knockout mice, and in humans we coined the term CF enteropathy after studying the intestinal mucosa with video capsule endoscopy which has been repeated by other researchers [58, 59]. On the molecular level, absence of cftr causes upregulation of oncogenic genes [89]. The inflammatory state in the gastrointestinal tract in CF patients promotes oncogenesis.

Epidemiological studies have suggested that CF is associated with an increased risk of gastrointestinal tract cancers. Colon cancer is the most common and represents the most significant risk. The risk was higher in male patients, those with PI and a history of DIOS [90]. Early detection of

colorectal cancer and removal of adenomatous polyps result in significant reduction in mortality. There is consensus endorsing population-wide screening for colon cancer beginning at 50 years of age. An excellent single-center study from Minnesota showed that in CF adults there is a fivefold increase in colonic neoplasms from the age of 40 [91]. The risk of CRC in patients with CF increases markedly after organ transplantation with the risk being over 25 times the age-adjusted baseline [92].

Yamada et al. have performed the first meta-analysis of the risk of gastrointestinal cancers in patients with CF compared to the general population [93]. The overall risk of gastrointestinal cancer was significantly elevated (SIR (standardized incidence ratio) 9.37) with significant increase in transplanted patients as well. The SIR of site-specific cancers of small bowel, colon, biliary tract, and pancreas was elevated.

The marked increase in the incidence of CRC prompted the North American CF Foundation to set up a Task Force whose findings have been published [94]. Colonoscopic screening for CRC should begin at 40 years of age with rescreening every 5 years. In patients who have had adenomas detected, this is increased to every 3 years. In transplanted patients, screening should begin at 30 years of age within 2 years of transplantation.

The emergence of gastrointestinal cancer as a potentially significant clinical problem in adult CF patients is a direct consequence of the greatly improved life expectancy. There is a possibility that chemopreventive drugs and the new CFTR modulator drugs (see below) which increase the expression of CFTR protein at the apical surface may also decrease the incidence of gastrointestinal cancers.

However, the meta-analysis has shown that CF should be added to the list of gastrointestinal cancer syndromes deserving special considerations for screening and surveillance [95].

Nutritional Complications

Malnutrition and lung disease are interrelated [96]. Care for CF involves attention to nutritional status to promote the most favorable outcomes. An experienced dietitian in the field of CF would play an essential role in the care of CF patients and should be an integral part of the CF team.

Nutritional Intake

A good nutritional diet regime is associated with improved lung function parameters, thus offering parents and patients with CF more motivation to adhere to prescribed therapies such as enzymes, vitamins, and a balanced, high-energy dietary intake. Studies from the 1980s changed the face of nutritional management of CF and proved that high-fat and high-energy diets were far superior to the old low-fat diet routines [97]. A diet that is composed of 35–40% of calories from fat is recommended in order to meet the energy demands of those with CF.

This diet regime given to infants from a neonatal screening program has demonstrated that normal growth can be achieved into adolescence. Farrell et al. found that the anthropometric indices of nutrition were significantly better in screened subjects compared with non-screened CF subjects up to the age of 16 years [98]. Recommendations by the North American Cystic Fibrosis Foundation Consensus Committee on Nutrition and European Cystic Fibrosis Society include appropriate evaluation of nutritional status at all ages and a diet that is age appropriate, with sufficient energy to meet needs for normal growth and weight gain [99, 100].

Feeding guidelines for infants with CF recommend breast milk as the primary source of nutrition for the first year of life [101]. Breastfeeding has been shown to be protective for the infant with CF. Breast-fed CF infants compared to formula had improved lung function with a reduced occurrence of infections during their first 3 years. Breast milk can provide the complete nutritional support for infants with CF during for the first 4–6 months. It is proven to be more efficient if supplemental energy is added to fortify a portion of the breast milk feeds with formula or by fortifying formulas to a more concentrated energy level for those infants on a combination of breast milk and formula or on formula alone. If breastfeeding is not an option or if supplementation is required, regular cow's milk-based infant formulas can be used [32]. In most cases, there is no need for a predigested formula. In warmer climates, sodium supplementation may often be recommended if reported to have a higher degree of loss in sweat. A total of 2–4 mmol/kg supplementation of milk or solids with table salt to provide per day is recommended; however, due to potential for errors in the accuracy of the measurement of the salt, liquid mineral mix solutions prepared by hospital pharmacies are often advised. Breast milk or formula intake should be supplemented with complementary solids after the first 4–6 months for increased energy. For the exclusively breast-fed infant, meat may be recommended as the first food due to its increased energy content and more importantly for its iron and zinc content. At 1 year of age, whole milk is recommended unless breast milk is continued.

Toddlers and Children

As the dietary intake and degree of physical activity vary with preschool-age toddlers and children, the addition of calorie-rich food is considered important. Self-feeding skills,

habits, and food preferences are established at this stage. Mealtimes should be a positive experience. School activities may conflict limiting snacks and adherence to enzymes; it is recommended that the health-care providers inform parents of appropriate strategies to support compliance with enzyme therapy.

Older children require higher nutrient intake due to acceleration of their growth; unfortunately, the progression of lung disease may compromise the nutritional status, as it increases the energy demands, interfering with appetite and resulting in decreased energy intake. If oral intake is not enough to support expected growth or nutritional status, a more aggressive nutritional intake via enteral tube feeding may be required and is best presented as a positive approach to help improve quality of life and health. However, a thorough evaluation of the nutritional failure must be completed before this type of support is implemented. The following should be considered and addressed as part of the evaluation: Behavioral and emotional issues, compliance, medical complications including GERD, cystic fibrosis-related diabetes (CFRD), or distal intestinal obstruction syndrome (DIOS) should be considered. Enteral tube feeding may provide approximately 30–50% of estimated daily energy requirements with appropriate enzyme therapy, generally delivered as overnight feed. The decision varies from individual patients as to when supplemental enteral nutrition is to be commenced. Some centers use intravenous lipids as an adjunct to enteral nutrition particularly in patients with low serum fatty acid concentration [102]. If total parenteral nutrition is used in non-post-surgical situations, weight gain is usually not sustainable once this support ceases.

The importance of the clinical staff being made aware of the overall nutritional status of their patient population allows for more focus on nutritional support. The sharing of these results with patients and families provides for teamwork and collaboration opportunities.

Fat-Soluble Vitamins

The neonatal diagnosis via screening programs is well documented. In non-screened populations, overt deficiencies are well described including benign intracranial hypertension [103], night blindness [104], rickets [105], hemolytic anemia [106], and coagulopathy due to vitamin A, D, E, and K deficiencies, respectively. Due to ongoing mild to moderate fat malabsorption, fat-soluble vitamin supplements are required for CF PI patients [107]. The European Guidelines are similar to those of North America, with the exception of a higher recommended vitamin A dose (4000–10,000 IU) and a higher starting dose of vitamin E and vitamin K (100 IU and 1 mg). This discrepancy is due to the lack of controlled studies that aid in defining lower limits of intake that support normal serum levels. For example, those patients who are PS, who do not require pancreatic enzyme supplementation for normal growth, have normal serum vitamin blood levels, and they do not require vitamin supplementation. There is some evidence that supplementation of fat-soluble vitamins in PS patients may be associated with decreased incidence of pulmonary exacerbations possibly due to the antioxidant effect of these compounds [108]. The North American CF Foundation Committee on vitamin D guidelines [109] advises higher supplemental amounts of vitamin D than in the CF vitamin supplements currently available for individuals with suboptimal serum 25-hydroxyvitmain D levels. Therefore, additional vitamin D supplements are recommended with the annual monitoring of serum vitamin levels for vitamins A, E, and D. Vitamin K may be difficult to monitor as the test used to assess serum levels, PIVKA (protein induced in vitamin K absence), is not routinely available. However, obtaining serum PIVKA or at least prothrombin levels are advised for those patients with hemoptysis or hematemesis and in patients with liver disease. More studies are needed in order to establish the minimum requirement of vitamin K [110]. The amount of vitamin K in vitamin supplements marketed to CF patients is unlikely to be sufficient. Toxicity of fat-soluble vitamins is very rare in CF; the one exception is post-lung transplantation (see below).

Bone Health

In CF patients, a decrease in bone mineral density (BMD) may begin in early childhood [111]. The nutritional status, serum calcium, vitamin D and K levels, pulmonary infection, exercise program, glucocorticoid medication, and class of CFTR mutation are all influencing factors of bone health. Poor BMD is a reflection of bone health [112]. Therefore, monitoring of BMD is recommended during routine visits.

Lung Transplantation

Patients with CF may present with added nutritional challenges pre- and post-lung transplant. Pretransplant, energy intake is often affected and most likely to be suboptimal, therefore requiring increased nutritional support via enteral tube-feeding support [113]. During pre- and post-lung transplantation, reduced BMD is a concern [114]. Post-transplant, patient's appetite usually improves, and oral intake is sufficient, with less and eventually no dependence on enteral tube-feeding support. Post-transplant, it is recommended to leave the enteral tube in place for a period of about 6 months to avoid insufficient oral intake to meet the energy needs and routinely blood monitor the fat-soluble vitamin levels. After transplantation, hypervitaminosis A has been reported,

although the etiology of this novel finding is unclear [115]. It may impact drug interactions, altered absorption, increased hepatic synthesis of retinol binding protein, or impaired retinol metabolism. The prescribed amount of vitamin supplements may need to be altered, as vitamin A levels may be elevated. Counseling patients that respond well post-transplant regarding lower energy food options is recommended as patients may experience an increased appetite due to the effects of corticosteroids and decreased rate of growth in children. These patients will benefit from an exercise and rehabilitation plan for lung performance and nutritional status [116].

Novel Therapies

Probiotics

There is increasing evidence that the CF gastrointestinal microbiome is altered, and that this dysbiosis contributes to disease manifestations in the GI tract [80]. One of the therapeutic strategies is to replace the "missing" elements of the microbiome. Probiotics, when administered in adequate amounts, may confer a health benefit on the host. Potential mechanisms of action include changes in respiratory and stool microbiome, thereby affecting respiratory and GI symptoms and systemic inflammation. A systematic review examined the effect of probiotics on respiratory, GI, and nutritional status in CF patients and showed a reduction in pulmonary exacerbations with an improvement in subjective GI symptoms [117]. Two studies measured the effect of probiotics on fecal microbiota composition and showed a significant increase in the proportion of Bacteroidetes and Firmicutes [118] and a significant increase in Bacteroides [119]. In a pilot study from the ESPGHAN Working Group, probiotic supplementation did not influence fecal calprotectin, pulmonary function, pulmonary exacerbations, and microbiome. However, 13% of patients normalized their gut permeability which requires further study [120].

Coffey et al. have performed a Cochrane study on probiotics in CF and concluded that there is some evidence that probiotics improve laboratory markers of intestinal inflammation but have not been proven to affect respiratory function [121].

Characterization of the microbiome in CF will enable personalized medicine. Strategies of restoration of a beneficial microbial structure or function could potentially offer novel treatment options.

CFTR Correctors and Potentiators

The emergence of novel targeted agents, which directly correct CFTR, has created new treatment opportunities for patients with cystic fibrosis. There are two main types of new treatment modulators and potentiators which are in clinical use for class II and III mutations.

The first drug is Ivacaftor, a CFTR potentiating drug which was successful in treating class III mutations. Remarkably, this drug improved respiratory function and decreased sweat chloride concentration to near-normal levels and caused a substantial weight gain [122].

The second drug was a combination of Ivacaftor and Lumacaftor for the more common class II mutations [123], and recently a triple combination of Ivacaftor, Lumacaftor, and Tezacaftor has shown impressive improvement in the most common class II mutation F50 del homozygote and heterozygote patients [124, 125].

The increase in weight cannot be explained by the improvement in respiratory function alone leading to speculation that these drugs also improve the function of the gastrointestinal tract. There is increasing evidence that there are direct effects of the modulator drugs on the gastrointestinal tract [126]. There is increasing evidence that exocrine pancreatic function improves in patients with class III mutations taking Ivacaftor with the intriguing notion that if treatment is started early enough, exocrine pancreatic function may be preserved [127, 128]. Many patients carrying class III mutations suffer from recurrent pancreatitis and this is markedly reduced on Ivacaftor therapy [129].

Ooi et al. have shown that treatment with Ivacaftor reduced stool calprotectin significantly and changes in the microbiome (increased presence of Akkermansia spp. and decreased Enterobacteriaceae spp.), suggesting an improvement in intestinal inflammation [130].

The availability of these novel drugs will enable research into specific disease processes affecting the liver, intestine, and exocrine pancreas in CF.

In conclusion, children and adults with CF have a wide range of gastroenterological manifestations. Gastroenterologists must be involved to improve the quality of life and outcomes of these patients.

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Small Intestinal Bacterial Overgrowth

42

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Introduction

Small intestinal bacterial overgrowth (SIBO) is a heterogenous disorder characterised by an excessive growth of select microorganisms within the small intestine. This excessive bacterial biomass, in turn, disrupts host physiology in a myriad of ways, leading to gastrointestinal and non-gastrointestinal symptoms and complications [1]. SIBO is a common cause of nonspecific gastrointestinal symptoms in children, such as chronic abdominal pain, abdominal distension, diarrhoea, and flatulence, amongst others [2–5]. In addition, it has recently been implicated in the pathophysiology of stunting [6], a disease that affects millions of children worldwide. Certain risk factors, such as acid-suppressive therapies [7–10], alterations in gastrointestinal motility and anatomy [11–20], and impoverished conditions [21–26], have been shown to predispose children to SIBO. Despite the relatively high prevalence of SIBO in children, it remains a poorly understood disorder. In fact, only a small number of studies have been published in the last two decades. Due to the relative scarcity of available literature and the recent emergence of novel, informative data regarding the underpinnings of the disease, an update for practicing paediatricians is now warranted. This chapter aims to comprehensively outline the most current information on SIBO in children, with particular emphasis on recent progress in our understanding of gut microbiota.

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The Gastrointestinal Tract and Gut Microbiota

Within the lumen of the gastrointestinal tract reside gut microbiota, who represent a complex and susceptible microbial consortium composed of enteric-adapted bacteria, acquired in the very early stages of life and developing in concert with the host [27, 28]. This mutualistic homeostasis is achieved through a range of mechanisms which maintain a life-long symbiotic microbe-host relationship, providing a plethora of important functions: from vitamin and short-chain fatty acid (SCFA) production to immunoregulation and neuropeptide secretion (for review see Ref. [29]).

The composition and functionality of gut microbiota are shaped and influenced by a multitude of intrinsic and extrinsic factors, such as genetics, mode of delivery, gestational age, feeding type and diet, pharmaceuticals, and even exercise [29]. The composition of gut microbiota exhibits variations related to the anatomical location in the gastrointestinal tract, which is, in turn, influenced primarily by factors such as luminal oxygen concentration and pH as well as the presence of bile and digestive enzymes. Indeed, bacterial numbers increase from about 10^3 to 10^4/mL in the stomach to approximately 10^{11}/mL in the colon [30–32]. Colonic microbiota represent the largest prokaryotic community in the human body, representing nearly 0.3% of the overall host body weight [33]. Although composed largely of four bacterial phyla – Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria – the infantile gut microbiome is dominated by Actinobacteria, with Firmicutes emerging to dominate after infancy [34]. In general, bacteria from the Actinobacteria (e.g. *Bifidobacterium*), Firmicutes (e.g. *Faecalibacterium*, *Clostridium*, *Ruminococcus*, *Lactobacillus*), and Bacteroidetes (e.g. *Bacteroides*, *Prevotella*) phyla are largely regarded as commensal microorganisms, while a significant portion of gastrointestinal pathogens belong to the Proteobacteria phylum (e.g. *Escherichia*, *Shigella*, *Salmonella*, *Klebsiella*, and *Helicobacter*) [35, 36]. In

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addition to this diverse community of bacteria, the human gastrointestinal tract is also home to an extensive array of viruses [37], fungi [38], and archaea [39]. While it remains unclear at present as to whether the former two kingdoms play a significant role in SIBO pathogenesis, methanogenic archaea, such as *Methanobrevibacter* spp., have been directly implicated in a methane-specific form of the disorder [40].

It is important to note, however, that despite the substantial advances made in the study of gut microbiota, small bowel microbiota remain poorly understood [41, 42]. Whereas colonic microbiota are more easily accessible and can be sampled via colonoscopy or faecal samples [43], sampling of small bowel microbiota poses a major challenge due to the invasiveness of the procedures (upper endoscopy) involved and the technical difficulties associated with these (such as avoiding contamination by oral microbiota). However, it appears that gut microbiota from stool samples and that from small bowel (sampled directly from small intestinal stomas) correlate well according to a small study of children with short bowel syndrome, particularly in relation to bacterial distribution, if not bacterial load [44]. The REIMAGINE (Revealing the Entire Intestinal Microbiota and its Associations with the Genetic, Immunologic, and Neuroendocrine Ecosystem) study [45] is an ongoing initiative that sets out to investigate the small bowel gut microbiome in health and disease.

Epidemiology in Children

The prevalence of SIBO in children has been explored in a wide spectrum of clinical contexts, including children living in impoverished conditions, individuals with chronic abdominal pain (CAP), as well as those who suffer from irritable bowel syndrome (IBS), stunting, and obesity, amongst other diseases. SIBO prevalence ranges from about 9% in children taking proton pump inhibitors (PPIs) [7] to approximately 90% in those with stunted growth [6] and chronic abdominal pain (CAP) [2]. However, it should be noted that data on the epidemiology of SIBO in children is limited by the small number of studies available, the lack of appropriate controls in some studies, and the varying test methodology employed and diagnostic cut-offs applied.

Pathogenesis

SIBO can negatively impact the host in a range of ways [1, 30, 46–54]. These include bacterial carbohydrate fermentation leading to excess gas production and fluid secretion [1]; bacterial deconjugation of bile acids resulting in poorly absorbed liposoluble vitamins as well as stimulation of colonic secretion and motility [18]; consumption of macronutrients and micronutrients by bacteria (bacterial-host nutrient competition), leaving the host with less available nutrients for absorption [51]; villous blunting leading to carbohydrate malabsorption [13, 55–57]; decreased short-chain fatty acid production [6, 58]; intestinal and systemic inflammation [16, 25, 59]; and increased gut permeability (Fig. 42.1). It should be noted that a recent systematic review [46] and clinical study [25] found conflicting and contradictory evidence with regard to effects on gut permeability.

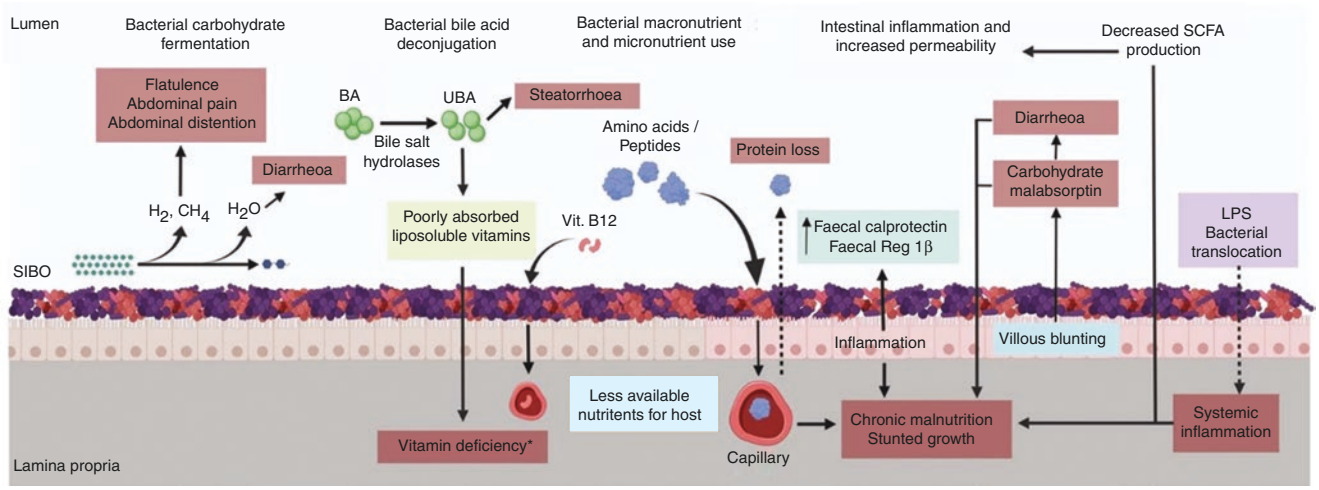

Fig. 42.1 Mechanisms through which SIBO affects the host. *The dotted arrows indicate increased intestinal permeability. * Includes vitamins A, D, E, and B_{12}. Vitamin K is synthesised by the gut microbiota, and thus its deficiency in this context is very unlikely. BA, bile acids; UBA, unconjugated bile acids; LPS, lipopolysaccharides*. (Created with BioRender.com. Developed under a CC BY 4.0 license and previously published in Front Pediatr. 2019 Sept 4;7:363)

Risk Factors

Acid-Suppressive Therapies

Gastric acid plays a crucial role in preventing pathogens from colonising the human alimentary tract, particularly the proximal portions [60]. Several extrinsic and intrinsic factors are known to alter this natural barrier [61], with one of them being proton pump inhibitor (PPI)-induced hypochlorhydria. PPIs are commonly prescribed for the treatment of gastroesophageal reflux disease (GERD) and, sometimes, for gastroesophageal reflux (GER) in children [62]. These drugs decrease gastric acid secretion by blocking the enzyme H^+/K^+-adenosine triphosphatase located in the apical membrane of parietal cells [63]. It has been estimated that approximately 34% [62] of paediatric patients treated with PPIs develop adverse effects, including infectious diarrhoea, *Clostridium difficile* infection, respiratory infections, and SIBO [64–70]. In addition, PPIs have also been shown to alter faecal gut microbiota of children and adults [71, 72]. In theory, the resultant acid suppression creates a more favourable environment for bacteria to overgrow, particularly Gram-positive, aerobic bacteria [30], leading to SIBO. However, a recent observational study [6] found contradictory evidence: despite the high prevalence of SIBO seen in the patient cohort (i.e. 96%), gastric pH was found to be in the acidic range (i.e. pH 2.7). It is important to note that pH measurements were only obtained from the study group and lacked normal reference values for comparison.

To date, four studies have assessed SIBO risk in children on acid-suppressive therapies [73]. In a study of children taking PPIs [7], of whom 77% took PPIs for over 12 months, SIBO was detected in 8.9% of children taking a PPI and in 3.7% of controls. In another study [10], the glucose hydrogen breath test (GHBT) was performed before and after a 3-month trial of omeprazole treatment in children with histologically proven peptic esophagitis, with 22.5% of children developing SIBO. Moreover, Rosen et al. [8] cultured the gastric fluid of children taking PPIs for at least 4 weeks and whose last dose was taken within 24 hours of sampling. Compared with the control group, the PPI group was found to have a significantly higher prevalence of gastric overgrowth (46% vs 18%), which was mainly caused by potential pathogens such as *Staphylococcus* and *Streptococcus*. In a double-blind, placebo-controlled randomised clinical trial [9], children were randomly assigned to one of two 4-week treatment groups: omeprazole/placebo group or omeprazole/probiotic. SIBO was excluded at baseline in all. Following the 4-week intervention, a second GHBT indicated that SIBO was present in 33% and 26% of omeprazole/probiotic group and omeprazole/placebo group, respectively.

Two meta-analyses [74, 75] of adult patients evaluated the association between PPI treatment and SIBO risk. In the most recent meta-analysis [74], 19 observational studies involving a total of 7055 adult subjects were included. Even though a high degree of heterogeneity was identified, the authors concluded that PPI therapy was associated with a modest risk for SIBO (odds ratio 1.71, 95% CI 1.20–2.43) after adjusting for study quality. The second meta-analysis [76] included 11 studies with a total of 3134 adult subjects. Likewise, the authors found a statistically significant association (OR, 2.282, 95% CI 1.238–4.205) between PPI therapy and SIBO, but only when the diagnosis was made with a "highly accurate testing modality" such as duodenal/jejunal aspirate and culture. It is important to note, however, that all the studies included in both meta-analyses were observational in nature and different diagnostic approaches (culture or breath tests) were used for SIBO diagnosis.

In conclusion, it appears that PPIs are a (perhaps weak) risk factor for SIBO, but only when this is assessed by culture-dependent approaches. The clinical implications are unclear given the overall low odds ratios (~2) and the lack of controls. Thus, until better data are available, it may be prudent to be judicious in prescribing long-term PPIs for children with GERD, and perhaps more importantly, that their use is avoided in children with GER [65, 67].

Intestinal Motility Disturbances

In addition to the acidic environment created by gastric acid, gut motility is critical to preventing SIBO. The migrating motor complex (MMC), a cyclic motor pattern that occurs during the interdigestive state, plays an important role in preventing the development of bacterial overgrowth within the small intestine [77]. In fact, the MMC is commonly referred to as the "intestinal housekeeper" given its role in this aspect of gastrointestinal health [78]. Under physiological conditions, the MMC sustains the aboral progression of luminal content in the small intestine between meals, thereby preventing stasis and SIBO. On this basis, an absent or disrupted MMC may lead to bacterial overgrowth [78].

The typical example of a disorder of gut dysmotility leading to SIBO is systemic sclerosis, with approximately 40% of adult patients being affected [47]. Despite the relatively high prevalence of gastrointestinal symptoms in children with juvenile systemic sclerosis (jSS) [79], to our knowledge, no studies have investigated the association of SIBO with jSS.

A somewhat more common example of gut dysmotility associated with increased risk of SIBO is cystic fibrosis (CF) [80]. Prevalence of SIBO in children with CF ranges from 30% [11, 81] to 56% [12] in different study cohorts. A study

[13] that used the HBT to evaluate orocecal transit time and SIBO in children with CF found that CF children had a significantly higher SIBO prevalence and the orocecal transit time was significantly longer than in healthy individuals. Data from animal studies showed that CF mice had a higher prevalence of SIBO than wild-type mice [82], which is thought to be due to slowed intestinal transit – possibly due to unabsorbed lipids leading to a triggering of the "ileal brake" – and/or smooth muscle dysfunction [80, 83], as well as mucus accumulation which acts as an anchor for bacteria, thereby facilitating overgrowth [76, 82]. Pancreatic insufficiency, a known risk factor for SIBO, might also play a role. Thus, the multifactorial nature of CF, including gut dysmotility, impaired mucus clearance, and a deficiency of pancreatic enzymes, seems to put children at a higher risk of developing SIBO.

Constipation has been shown to be associated with SIBO. However, the causative or consequential nature of this interaction is unclear. In theory, slower orocecal transit seen in constipation may fail to clear the luminal content, thereby increasing the risk of SIBO. On the other hand, methane, a biologically active gas produced instead of hydrogen by some individuals from bacterial fermentation of carbohydrates, can delay intestinal transit, which in turn may lead to constipation. In a study of children with myelomeningocele [14], SIBO was diagnosed in 39% (7/18), and, interestingly, all methane producers had a delayed orocecal transit time. Moreover, another study found a 42% (21/50) prevalence of SIBO amongst children with retentive faecal incontinence, of whom 8 had methanogenic SIBO, 11 had hydrogen-type SIBO, and 2 had mixed (methane and hydrogen)-type SIBO. In addition, 48% of patients with faecal incontinence were found to have high basal methane concentrations (>10 ppm) as compared with 10% of control subjects [15]. Furthermore, in a study of children with constipation [20], methane production was found in 73.5% (25/34) of children with constipation and soiling as compared with 1% of children with constipation alone.

The notion that a slowed intestinal transit may predispose to SIBO is supported by two recent studies in adults [84, 85]. In the most recent study [84], two patient groups (PPI group and PPI/prokinetic group) underwent GHBT and lactulose hydrogen breath test (LHBT) and evaluation of orocecal transit time. Interestingly, SIBO was more common in the PPI alone group than in the PPI + prokinetic group, and SIBO-positive patients had slower orocecal transit times than SIBO-negative patients. However, it is important to note that the overall SIBO prevalence was only 8.8% (13 subjects), and even though the authors measured both hydrogen and methane, it was not specified whether the three patients with methane-positive SIBO had normal or delayed orocecal transit times. Furthermore, in the second study [85], 29 female patients with functional constipation were prospectively enrolled in a 2-week trial of lubiprostone. Gastrointestinal transit and SIBO were evaluated by the wireless motility capsule (WMC) and lactulose hydrogen/methane breath tests, respectively. At baseline, 68% of patients had increased levels of both hydrogen and methane, suggesting SIBO. After treatment completion, 41% of patients became SIBO-negative, which was paralleled by a 30% increase in small bowel transit time. However, it is important to mention that the authors considered methane positivity as an increase of ≥3 ppm, which differs from the current guidelines in which methane positivity is defined as an increase of ≥10 ppm [86]. It is challenging to interpret these results for two reasons: (1) all patients had constipation and were methane producers at baseline (i.e. methane production of ≥3 ppm); thus, this suggests constipation may have been caused by intestinal methane production rather than constipation leading to SIBO; or simply, the methane values present in these patients could be physiological; and (2) the fact that lubiprostone "cured" 41% of patients suggests that by increasing intestinal transit (and thus intraluminal clearance), faecal stasis decreases, which might secondarily prevent the development of SIBO.

Anatomical Alterations

Anatomical boundaries, and particularly the ileocecal valve, are considered crucial for maintaining a harmonised microbial community within the human alimentary tract, Absence or dysfunction of the ileocecal valve has been shown to predispose patients to SIBO, as colonic bacteria are thought to be "backwashed" into the small intestine, thereby leading to coliform-type SIBO development [30].

Paediatric intestinal failure is a chronic disease variably characterised by altered intestinal motility and anatomy, absence of the ileocecal valve, and prolonged use of antacids and antibiotics, which increases the risk of SIBO [87, 88]. Over the past few years, a plethora of research has been conducted on SIBO in children with intestinal failure. Children with intestinal failure have high rates of SIBO, ranging from 40% [16, 18] to 78% [17, 19] in different study cohorts. Absence or dysfunction of the ileocecal valve [16, 18, 19], a shorter small bowel remnant [89], as well as use of parenteral nutrition (PN) [17, 90] are factors independently associated with an increased risk of SIBO in these children. Furthermore, children with intestinal failure and clinical SIBO appear to be more likely to develop ambulatory central line-associated bloodstream infection, possibly due to increased intestinal permeability and bacterial translocation [91]. An interesting study [92] that evaluated the faecal gut microbiota of children with short bowel syndrome found that those on PN had decreased diversity and increased numbers of Proteobacteria as compared with those who were weaned

from PN. None of the PN patients had an ileocecal valve, and, of these, four out five patients evaluated for SIBO were on with antibiotics, which may explain the observed microbiota perturbations.

Taken together, these findings demonstrate that paediatric intestinal failure is a multifactorial process with an increased risk of SIBO. Resection of the ileocecal valve, in particular, appears to be a common finding in children with intestinal failure and SIBO, strongly suggesting the role of this structure in SIBO prevention. In line with this, a study of adult patients [93] investigated the ileocecal junction pressure using the wireless motility capsule and the presence of SIBO by the LHBT and small bowel aspirate and culture. Interestingly, the authors found a combination of SIBO-predisposing factors in their patient cohort: (1) the small bowel transit time in SIBO patients was significantly slower than those without SIBO, (2) the gastric pH was significantly higher in SIBO patients than those without SIBO, and (3) the mean ileocecal junctional pressure was significantly lower amongst SIBO patients than those without SIBO. Thus, these findings reinforce the fact that an "incompetent" ileocecal valve predisposes to SIBO and emphasise the multifactorial nature of the disease.

Impoverished Conditions and Poor Socioeconomic Status

Impoverished conditions, which are commonly associated with a lack of basic sanitation services such as clean water, appropriate sewage, and collection of household garbage, may be a risk factor for SIBO. Six studies (one from Myanmar [21], three from Brazil [22–24], and two from Bangladesh [25, 26]) have investigated this association in children.

A study from Myanmar (in a Burmese village) [21] investigated the prevalence of SIBO in children using the LHBT. Around 85% of the village's population obtained drinking water from surface wells and ponds, and approximately 10% used rainwater for the same purpose [94]. SIBO was diagnosed in 27% of children (53/195), with males being more commonly affected than females. In another study [22], SIBO prevalence was significantly higher in children living in a slum compared with controls living in households with appropriate sanitation services (37.5% vs 2.1%, respectively). Another study from Brazil [23] documented significantly higher prevalence of SIBO in slum-dwelling children in comparison to their socioeconomically advantaged counterparts (30.9% vs 2.4%, respectively). Interestingly, the authors did not find statistically significant differences in the environmental variables (i.e. water contamination with coliforms, access to public water network, access to public sewage, and public collection of household garbage) between the SIBO-positive and SIBO-negative slum dwellers. In another study by the same group [24], 60% of children were found to have SIBO, and their faecal gut microbiota analyses showed a significantly higher number of *Salmonella* spp. and lower numbers of Firmicutes. Moreover, in a study of 90 Bangladeshi children belonging to the lowest socioeconomic strata [25], 16.7% had a positive GHBT; furthermore, the odds of developing SIBO were increased by the presence of an open drain/sewer outside the home (OR, 4.78; 95% CI, 1.06–21.62). In the same cohort of children [95], and using 16 S V4 rDNA to analyse faecal gut microbiota, a *Lactobacillus* bloom was noted in children with a positive GHBT. A recent study on the faecal gut microbiota of children living in urban slums in Mumbai, India, found a dominance of Proteobacteria [96]. Although the authors did not perform breath tests to diagnose SIBO, it is known that bacteria belonging to the Proteobacteria phylum are the most common causative agents of SIBO.

Taken together, these findings demonstrate that children living in poor sanitation conditions, particularly those involving exposure to contaminated water, may have a higher risk of developing SIBO and a disturbed gut microbiome. However, it is important to mention that none of these studies provided a clear pathophysiological mechanism through which such unsanitary conditions predispose children to the disease. It has been hypothesised that repeated exposure to abnormal levels of lipopolysaccharides found in soil and drinking water may disrupt the MMC, causing intestinal stasis (as seen in patients with gut dysmotility), thereby leading to SIBO [48]. However, while this hypothesis appears to be biologically plausible, to date, no studies have evaluated it.

Other Risk Factors for SIBO

Immunodeficiency and celiac disease (CD) have also been regarded as potential risk factors for SIBO. As for immunodeficiency disorders, a small-scale study [97] on children with immunodeficiency syndromes (i.e. IgA deficiency, hypogammaglobulinaemia, and T cell defects) found SIBO in 41% of patients via jejunal aspirate and culture, and the most common clinical manifestation was chronic diarrhoea. Moreover, a recent small study of adult patients with common variable immunodeficiency [98] found SIBO in 40% of the cohort, with co-occurrence of abdominal pain and anaemia being significantly more common in SIBO-positive than SIBO-negative individuals. In another study [99], 4% ($n = 12/296$) of young adults with chronic diarrhoea and malabsorption syndrome were found to have hypogammaglobulinaemia. Of these, 25% ($n = 3/12$) were diagnosed with SIBO. Although these findings show a relatively high prevalence of SIBO in children and adults with immunodeficiency syndromes, large-scale studies are needed in order to further support this association.

As for CD, a recent systematic review [100] of studies in adults with CD documented a 20% pooled mean prevalence of SIBO. The authors concluded that SIBO may be common in patients with CD who do not improve following institution of a gluten-free diet. Two studies characterised microbiota composition and diversity amongst children with CD [101, 102] and found that those with active CD had a higher abundance of members of the phylum Proteobacteria [101] and lower ecological indices of genus *Lactobacillus* [102] as compared to healthy individuals and CD patients in remission. Although these studies did not set out to evaluate SIBO per se and as such did not report CFU/g, the higher abundance of Proteobacteria and lower ecological indices of genus *Lactobacillus* seen in children with CD may indicate a disturbed microbial ecosystem. Certainly, a study that evaluates the presence of SIBO in children with CD, either by the H_2/CH_4 breath test or small intestinal aspirate and culture, would be of great interest.

Although less studied, SIBO has also been noted to be more prevalent in nutritionally deficient disease states including intestinal failure requiring parental nutrition [90] and cystic fibrosis [81]. It is important to note, however, that SIBO can develop even in the absence of any of the aforementioned risk factors. In line with the findings by Boissieu et al. [3] and our own experience, many children who test positive for SIBO are "healthy" and have no evident risk factors. There are clearly many questions to be addressed by future studies regarding the risk factors for SIBO in children.

Aetiology of Paediatric SIBO

SIBO can be caused by archaea or bacteria, by one or more microorganisms, by Gram-positive or Gram-negative bacteria, and by anaerobic or aerobic microorganisms [30]. In a cross-sectional study in which patients underwent duodenal aspirate and culture for the diagnosis of SIBO (diagnostic threshold of $>10^3$ CFU/mL) [103], SIBO was diagnosed in 20% of cases, being caused by one microorganism in 54.7% of cases and by two microorganisms in the remainder. In addition, the vast majority of bacterial isolates belonged to the phyla Proteobacteria and Firmicutes, with the most common being *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Citrobacter freundii*, *Serratia marcescens*, and *Enterococcus faecium* (in descending order of frequency). In another study that used the same diagnostic threshold [104], SIBO was diagnosed in 19.4% of adult subjects, and, again, most bacterial isolates were members of the phyla Proteobacteria and Firmicutes (*Escherichia coli*, *Enterococcus* spp., *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Citrobacter freundii*, and *Serratia marcescens*), and in 75% of cases the overgrowth was due to one microorganism. In the remainder, SIBO was caused by two microorganisms, with the most common being *E. coli/K. pneumoniae* and *E. coli/Enterococcus* spp. Furthermore, Gutierrez et al. [17] conducted a retrospective study on children with intestinal failure who underwent duodenal aspirate and culture. SIBO was diagnosed in 70% according to the more stringent diagnostic threshold of $>10^5$ CFU/mL. Again, the most common causative microorganisms were members of the phyla Proteobacteria and Firmicutes, with the most common being *E. coli*, *Streptococcus viridans*, *K pneumoniae*, *Enterococcus* spp., and *Pseudomonas aeruginosa*. The overgrowth was due to more than one bacterium in a minority of patients. Moreover, Galloway [18] diagnosed SIBO in 43% of children with intestinal failure, of whom five out of six patients had overgrowth due to two different microorganisms and only one had overgrowth caused by one microorganism, with *Enterococcus* and *Klebsiella* being the most frequently isolated bacteria.

In a more recent cross-sectional study of adult subjects with non-alcoholic steatohepatitis (NASH), and employing the diagnostic threshold for SIBO of $\geq 10^5$ CFU/mL and defining low-grade bacterial overgrowth as $\geq 10^3$ CFU/mL [105], 20% and 60% of cases are diagnosed with SIBO and low-grade bacterial overgrowth, respectively. Amongst the SIBO cases, Proteobacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Acinetobacter* spp.) were the most commonly isolated microorganisms, followed by members of the Firmicutes phylum (*Streptococcus* spp. and *Enterococcus faecalis*); 20% of cases were due to overgrowth of more than one microorganism.

Taken together, these findings demonstrate that in most cases, SIBO is caused by a single microorganism belonging to the phylum Proteobacteria [36], particularly coliform bacteria such as *E. coli* and *Klebsiella* spp. However, it is important to consider three aspects: (1) culture-dependent approaches were used in these studies, which means that there was a risk of missing bacteria that remain difficult to culture under clinical laboratory conditions; (2) only two studies were conducted on children [17]; and (3) SIBO was studied in a wide spectrum of clinical contexts, including IBS, intestinal failure, and non-alcoholic steatohepatitis. Thus, the specificity of such microbiota alterations for SIBO must be interpreted in the context of these potentially important confounders.

Next-generation sequencing (NGS) methods such as 16S ribosomal RNA (rRNA) sequencing provide a reasonably high-resolution and relatively cost-effective way to study the human gut microbiome [106–108]. Only one study in children [6] has used NGS to evaluate gut microbiota in the context of SIBO. Vonaesch et al. [6] conducted a novel, cross-sectional study on stunted and healthy children from

Madagascar and the Central African Republic, in which 16S rRNA amplicon sequencing was used to analyse the faecal gut microbiota composition of both groups and to confirm the presence of bacteria identified by gastric and duodenal fluid aspirate cultures. Despite the use of a higher diagnostic threshold for SIBO (i.e. $>10^5$ CFU/mL), SIBO was diagnosed in 96% of stunted children (Madagascar, 100%, and Central African Republic, 88%) and, interestingly, the most common causative microorganisms were oropharyngeal colonisers such as *Streptococcus* spp., *Staphylococcus* spp., *Haemophilus* spp., *Moraxella* spp., and *Neisseria* spp. In addition, microbiota sequencing revealed overrepresentation of oropharyngeal species (e.g. *Streptococcus* spp., *Haemophilus* spp., *Neisseria* spp., *Rothia* spp., *Actinomyces* spp., and *Gemella* spp.) and enteropathogens (e.g. *Escherichia coli*, *Shigella*, and *Campylobacter*), as well as underrepresentation of butyrate producers (e.g. *Clostridia* spp.) in stunted children compared with controls.

More recently, as part of the REIMAGINE initiative [45], a study was conducted on adult subjects who underwent upper endoscopy and whose duodenal aspirates were cultured and analysed by 16S rRNA sequencing [109]. The authors used a lower threshold of $>10^3$ CFU/mL for SIBO diagnosis. Compared with non-SIBO subjects, the α-diversity of the duodenal microbiome of SIBO subjects was decreased, and their predominant phylum was Proteobacteria (particularly Gammaproteobacteria), which was three-fold more abundant than in non-SIBO subjects, whose predominant phylum was Firmicutes. Again, these findings support the role of Proteobacteria as an important causative agent of SIBO.

Clinical Features and Complications

Paediatric SIBO is a heterogenous disorder that manifests itself through nonspecific symptomatology, including gastrointestinal and non-gastrointestinal symptoms [30, 110]. The most common signs and symptoms reported in the literature are chronic abdominal pain, abdominal distension, diarrhoea, flatulence, belching, steatorrhoea, fetid stools, mucus in stools, fatigue, nausea, and stunted growth [2–6].

SIBO and Functional Gastrointestinal Disorders

A small number of observational studies have evaluated the association between FGIDs and SIBO in the paediatric population.

In a study of children with abdominal pain-related functional gastrointestinal disorders (irritable bowel syndrome [IBS], functional abdominal pain [FAP], functional dyspepsia [FD], and FAP syndrome) [4], amongst the 14.3% SIBO-positive subjects (positive GHBT), the most common symptoms were fatigue (75%), altered defecation pattern (71%), nausea (68%), and bloating (66%). However, only altered defecation pattern, loss of appetite, and belching were significantly more common in SIBO-positive than SIBO-negative subjects; diarrhoea and flatulence did not reach statistical significance. In another study of children with chronic abdominal pain (FD, IBS, and FAP) and controls [111], 91% of cases and 35% of healthy controls had a positive breath test, respectively. Surprisingly, there were no differences in the presence of gastrointestinal symptoms, such as bloating, gas, incomplete evacuation, constipation, diarrhoea, mucus in stool, or straining, between SIBO-positive and SIBO-negative subjects; however, it is important to mention that only seven control subjects were included, in comparison to a very disproportional number of abdominal pain sufferers. Moreover, in a study that evaluated the prevalence of SIBO in children with IBS [112], SIBO prevalence was higher amongst those with IBS compared to their healthy counterparts (65% vs 7%, respectively). In a small retrospective study [113], children with chronic abdominal pain and atopy were more likely to have SIBO than non-atopic children and especially those with allergic rhinitis, asthma, and cow's milk protein allergy. Taken together, these findings suggest that SIBO is a frequent underlying diagnosis in children with functional abdominal pain disorders (i.e. IBS and FD), thus suggesting a role in their pathogenesis.

The IBS-SIBO interaction in adults has attracted a tremendous amount of research and controversy in the last decade. A systematic review and meta-analysis [114] of observational studies in adults estimated the prevalence and determined predictors of SIBO in IBS. The authors found an overall pooled SIBO prevalence of 38% (95% CI 32–44) as well as a 4.7 (95% CI 3.1–7.2) pooled OR for SIBO in IBS subjects as compared with healthy controls, and surprisingly, PPI use was not associated with SIBO. It should be noted that there was significant clinical and test modality heterogeneity between these various studies [115]. Even though a growing body of literature suggests an association between the two disorders, it is unclear whether SIBO precedes IBS or vice versa [116, 117]. Thus, until better data are available, children presenting with IBS-like symptomatology may merit evaluation for SIBO, such as by a H_2/CH_4 breath test. The role of SIBO role in IBS is comprehensively reviewed elsewhere [54, 118].

Constipation, another FGID, has been associated with intestinal methane production in adults and children (methanogenic SIBO) [119]. A 2011 meta-analysis [120] of nine studies (1277 subjects) found a significant association between methane production on a breath test and constipation (OR 3.51, CI 2.00–6.16). In a study of animal and human

models [121], intraluminal infusion of methane reduced small bowel transit by 59% in canine models as compared with controls, and methane was also found to increase intestinal contractile activity in guinea pigs and in patients with IBS. In contrast, in a study of children living in a slum [23], the authors did not find an association between methane and constipation in the patient cohort; in fact, none of these children had constipation despite a 30% prevalence of methanogenic SIBO. In another study [122], the effect of rifaximin on breath methane and colonic transit in adult patients with constipation was investigated. The authors found that a larger percentage of patients with chronic constipation were methane producers (>10 ppm) and had slower colonic transit times as compared with controls. Methane producers ($n = 13$) were randomly assigned into two groups: rifaximin group (14-day trial) and placebo group. After treatment, the rifaximin group had a significantly lower area under the curve for methane production and colonic transit time normalised in 66% of cases as compared with the placebo group, in whom colonic transit time did not normalise. Thus, these findings support the association between methanogenic SIBO and constipation. The most important question to be answered is whether it would be cost-effective to perform methane breath tests on all children with "functional" constipation, considering the relatively high prevalence of the disorder. What we know from small-scale studies is that children with constipation and retentive faecal incontinence are more likely to be methane producers than children with constipation alone [15, 20]. These children may benefit from antibiotic therapy. However, large-scale, prospective studies are warranted in order to (1) clarify the relationship between methanogenic SIBO and constipation and (2) determine whether children with methanogenic SIBO and constipation benefit from antibiotic treatment.

In a recent retrospective study in a cohort of children with various gastrointestinal manifestations and feeding difficulties [123], 26% of children were diagnosed with SIBO by duodenal aspirate culture (>10^5 CFU/mL), and interestingly, 50% of these were also found to have lactase deficiency on analysis of enzyme activity, possibly due to villous blunting.

SIBO and Systemic Disorders

Increasing evidence suggests that SIBO may be implicated in the pathogenesis of stunted growth [6, 25] and environmental enteric dysfunction (EED; formerly known as environmental enteropathy and, in the past, as tropical sprue) [24, 48, 49, 53]. Approximately 20% of children under five suffer from poor linear growth (also known as stunting) worldwide [124]. Intraluminal competition for micro- and macronutrients between the excessive bacterial biomass and the host [125], as well as other SIBO-induced factors such as diarrhoea, carbohydrate malabsorption, protein loss, increased intestinal permeability, and intestinal and systemic inflammation, may lead to a negative caloric balance in the host, thereby resulting in stunted growth and malnutrition. Such factors, too, characterise EED; thus, it appears that SIBO may play an important role in both EED and stunted growth [6, 126]. In a study conducted in Madagascar and the Central African Republic [6], nearly all of the cohort of stunted children was diagnosed with SIBO (i.e. 96%), and another study from Bangladesh [25] also found an increased prevalence of SIBO at 16.7% in stunted children. However, research from an impoverished community in Zambia, Africa [127], failed to demonstrate an increased prevalence of SIBO in malnourished children, with only four children (8%) having a positive breath test. A retrospective study that included data from 162 children found that children with a positive HBT had significantly lower heights and weights than those with a negative HBT [128]. In a different study [96], the faecal gut microbiome of a cohort of undernourished children from Mumbai, India, was characterised by a Proteobacteria dominance, despite the fact that most children were breastfed. Taken together, these findings suggest a possible role for SIBO in the pathogenesis of stunting. Thus, it may be reasonable to perform a hydrogen/methane breath test as part of the clinical approach to children with poor linear growth.

Although SIBO has been widely described amongst undernourished children, emerging evidence suggests that, at the opposite end of the nutritional spectrum, children with excessive adipose tissue also seem to have high rates of SIBO. In a recent prospective study of overweight and obese children, almost 2/3 of the cohort had a positive LHBT, but all children exhibited increased inflammatory markers, including non-SIBO children, suggesting that SIBO may be an incidental rather than a causative factor in overweight and obese children [129]. Despite this, a clear association appears to be emerging between SIBO and non-alcoholic fatty liver disease (NAFLD). A recent meta-analysis found that NAFLD patients had an overall 2.8 relative risk of SIBO compared to healthy control cohorts [130]. In a cohort of 89 overweight and obese children, significantly higher rates of liver steatosis were uncovered in those with SIBO (70.9%) compared to those without (41.4%) [131]. In addition, the children affected by SIBO had higher average glucose levels and homeostatic model assessment (HOMA) indices, reflecting poorer glycaemic control and insulin intolerance. Similarly, in a case-control study of 30 overweight/obese children with SIBO and 28 overweight/obese children without SIBO, it was found that liver steatosis was more than twice as prevalent in the former population (70% vs 32%, respectively) [132].

Moreover, although deficiencies of liposoluble vitamins (A, D, and E) and vitamin B_{12} have been documented in adults [133, 134], no studies have explored this in children with SIBO. However, in a recent, small study of children with short bowel syndrome [135], those who were being treated for SIBO with metronidazole (5/13) had persistently

elevated levels of methylmalonic acid, suggesting lower levels of vitamin B_{12}. Menaquinone (vitamin K2) is produced by gut microbiota [136], and thus, from a physiological standpoint, it would seem counterintuitive to suggest that vitamin K deficiency would arise in a bacterial-abundant environment. Nonetheless, a recent case report described a 17-year-old female with vitamin K deficiency possibly caused by SIBO. The authors speculated that the vitamin K deficiency seen in this patient may have been the result of reduced menaquinone-producing bacteria, expansion of vitamin K-consuming bacteria, or severe malabsorption [137]. Further gut metabolome studies [138] are needed to elucidate SIBO's role in vitamin deficiencies.

Currently, there are no guidelines on the diagnosis or treatment of SIBO in children. Given the heterogenous and nonspecific nature of the disease, it is challenging to decide in whom to initiate an evaluation for SIBO.

Diagnosis

SIBO can be diagnosed by invasive and non-invasive methods. The non-invasive methods include breath tests, while invasive methods comprise culture-dependent and culture-independent approaches.

Hydrogen and Methane Breath Testing

Although subject to debate and controversy [139–141], H_2 and CH_4 breath tests are increasingly being used due to their availability in healthcare facilities; because they are inexpensive, easy to use, and non-invasive (which is extremely important in paediatrics); and because the results can be interpreted on the same day of the test (the H_2/CH_4 breath test procedure is thoroughly reviewed elsewhere [86, 142, 143]). According to the 2017 North American Consensus [86], the diagnosis of SIBO is *suggested* when there is an increase in H_2 of ≥20 ppm over baseline within the first 90 minutes of the test with either lactulose or glucose, or when there is an increase in CH_4 of ≥10 ppm at any time point during the test (Fig. 42.2). On the other hand, the older Rome Consensus [143] recommended the use of glucose as substrate due to its greater accuracy and defined SIBO as an increase in H_2 of ≥12 above baseline by using the GHBT.

In order to understand the mechanisms behind H_2 and CH_4 breath testing, one must be aware of two concepts: orocecal transit time and intraluminal gas production. The human body has no means of producing H_2 and CH_4 other than through intraluminal microbial fermentation and methanogenesis, respectively [86, 143–145]. As previously mentioned, colonic microbiota are predominated by fibre-fermenting anaerobes, mainly those belonging to the phyla Actinobacteria and Firmicutes [146]. Undigestible carbohydrates reaching the colon are readily fermented by hydrogenogens (H_2-producing bacteria) [147], and hence a physiological increase in H_2 is expected in the colon. Intraluminal H_2, in turn, is absorbed into the systemic circulation and is transported to the lungs where it can then be released through exhaled breath [142]. Moreover, the orocecal transit time – the elapsed time between ingestion of a substance until it reaches the cecum – has been shown to be around 90 minutes in both adults and children, as assessed by the LHBT [148]. Thus, in the presence of SIBO, substrate administration will result in a small bowel H_2 peak, occurring before the orocecal transit time has completed (i.e. 90 minutes).

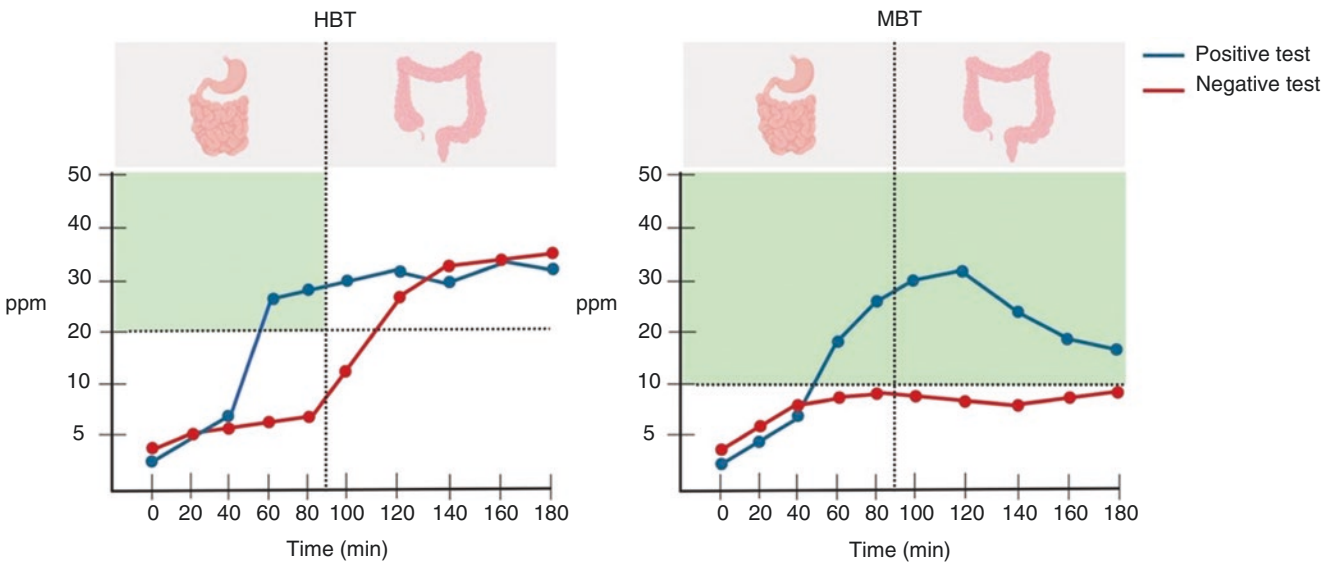

Fig. 42.2 Graphical representation of the hydrogen and methane breath tests. *The vertical dotted line indicates the completion of the orocecal transit time, and the horizontal dotted line indicates the current diagnostic thresholds for SIBO. The green shaded areas indicate where the test is considered positive*. (Created with BioRender.com. Developed under a CC BY 4.0 license and previously published in Front Pediatr. 2019 Sept 4;7:363)

Glucose and lactulose are the substrates usually used in breath tests to diagnose SIBO. The former is a monosaccharide which is readily absorbed in the proximal small intestine, and the latter is a synthetic, undigestible disaccharide that reaches the cecum intact. In the presence of SIBO, glucose administration will result in a H_2 peak which is produced in the small intestine (i.e. <90 minutes); in addition to this peak, lactulose administration can give rise to a second – colonic – H_2 peak: the early peak produced by bacterial overgrowth in the small intestine and the second one caused by colonic fermentation [86, 142]. However, both substrates have their own advantages and limitations. By using glucose, false-negative results can arise in the presence of bacterial overgrowth in the distal small bowel (i.e. ileum), as glucose is readily absorbed proximally. In addition, accelerated orocecal transit can give rise to false-positive results as the substrate reaches the colon rapidly, thereby undergoing premature fermentation by colon bacteria – this can occur with both glucose and lactulose [139]. False-negative results can also arise in the absence of H_2-producing bacteria or in the presence of CH_4-producing microorganisms (discussed below) [143]. Based on a 2008 systematic review [149], the sensitivity and specificity of the GHBT ranged from 20% to 93% and 30% to 86%, respectively, and the sensitivity and specificity of the LHBT from 31% to 68% and 44% to 100%, respectively. The most recent systematic review and meta-analysis [150] found a pooled sensitivity and specificity for the GHBT of 54.5% and 83.2%, respectively (positive likelihood ratio 2.45 (95% CI, 1.51–3.97), and negative likelihood ratio 0.60 (95% CI, 0.45–0.80)). As for the LHBT, the pooled sensitivity and specificity were 42% and 70.6%, respectively (positive likelihood ratio 1.30 (95% CI, 0.77–2.22) and negative likelihood ratio 0.79 (95% CI, 0.57–1.08)). It is important to mention that study heterogeneity was identified in almost all of the variables included. The authors concluded that the GHBT is the preferred method given its higher diagnostic yield. Moreover, in a study by the REIMAGINE group [45], the sensitivity and specificity of the LHBT were 57.14% and 84.61%, respectively, in subjects diagnosed with SIBO based on the $>10^3$ CFU/mL threshold. However, this study was limited by the small number of patients who underwent breath tests.

Methanobrevibacter smithii, a member of the domain Archaea and the most abundant methanogen in the human alimentary tract, produces CH_4 as an end product of hydrogen metabolism (hydrogenotrophic methanogenesis), by using one molecule of carbon dioxide and four molecules of H_2. The archaeon is extremely oxygen sensitive and relies completely on hydrogenogens for CH_4 production, due to its inability to metabolise monosaccharides. For this reason, *Methanobrevibacter smithii* is considered an "obligate cross-feeder" [144]. Furthermore, since the archaeon utilises H_2 to produce CH4, it can lead to a falsely negative HBT. Thus, it is recommended that both H_2 and CH4 are measured in order to avoid this [86].

In conclusion, despite the limitations of the H_2/CH_4 test [141], it remains a useful tool in the diagnosis of SIBO in children due to its practical and non-invasive nature. Importantly, there are no reported significant side effects for the hydrogen/methane breath test other than transient abdominal pain and/or vomiting during the test.

Culture-Dependent Approaches

Culture-dependent approaches are considered the current gold standard for definitive SIBO diagnosis. A small intestinal (i.e. duodenum or jejunum) aspirate is obtained at upper gastrointestinal endoscopy through a sterile catheter, or alternatively via capsule or antegrade double-balloon endoscopy [151], and stored anaerobically prior to culture for both aerobic and anaerobic bacteria on media such as MacConkey's or blood agar [109]. When based on the culture of a proximal jejunal aspirate, a bacterial concentration of $>10^3$ CFU/mL is regarded as indicative of SIBO [86, 110], although there is some heterogeneity in the literature in this regard and some experts recommend a threshold of $>10^5$ CFU/mL as more specific [152].

Despite being regarded as the gold standard, there are several notable limitations to this approach. Firstly, endoscopy-guided aspiration is invasive, is expensive, and requires specialist input, limiting its availability and application in certain settings. Secondly, we know from microbiome research that standard clinical laboratory culture-based approaches have the potential to detect only a minority of the extant bacterial consortium. In addition, care must also be taken not to contaminate samples or sampling apparatus with oropharyngeal microbes, thereby contributing to false positives. Likewise, the significant heterogeneity in aspirate sampling protocols impacts substantially on test outcome and accuracy. For example, one study previously used air, rather than nitrogen or carbon dioxide, during endoscopy to recover aspirate samples in suspected SIBO and, not surprisingly, then went on to culture anaerobic bacteria from just 1 of 50 samples [153]. Other variables include the specific site of sampling and volume of aspirate. The lack of standardised protocol and diagnostic consensus for what has been long regarded as the gold standard undoubtedly contributes to the massive variation in SIBO prevalence and incidence rates reported in the literature. This, in turn, limits comparability between studies and ultimately hinders research progress in the field. There is an urgent need for standardisation and validation of protocols and diagnostic criteria. Ethical constraints on the performance of endoscopy on healthy control children continue to present an obstacle to the vali-

dation of normal values for aspirates; therefore, the development of accurate less invasive diagnostic approaches assumes considerable urgency.

SIBO in the Era of Next-Generation Sequencing

As alluded to previously, culture-independent approaches based on molecular technologies have become the most widely applied modalities for studying both the composition and metabolic activity of gut microbiota. There are several considerations to be addressed when selecting a methodology, including sample type, DNA extraction method, sequencing modality and platform, as well as bioinformatic pipeline (reviewed comprehensively in Ref. [43]).

In general, the composition of gut microbiota is currently most cost effectively studied through 16S rRNA sequencing. The 16S region is a highly conserved region of rRNA within all taxa of bacteria, which displays sufficient variation and divergence in sequence to allow differentiation at the genus level. Although it has been central to progress in the field of microbiome research, 16S rRNA sequencing is crucially limited by the fact that it does not generate absolute data on quantities of bacteria, but rather provides investigators with a relative abundance of taxa within a sample. The second modality which is indeed worthy of consideration is metagenomic shotgun sequencing. While 16S rRNA sequencing provides an overview of microbiota composition, metagenomic shotgun sequencing goes a step further by informing us of both who is there and of what are they metabolically capable of.

Although such sequencing capabilities are now commonplace in microbiological and medical research laboratories, uptake and application to SIBO research have been comparatively slow, and, as a result, there is a dearth of relevant clinical data available. Despite this, several clinical studies which targeted alternate, but related, gastrointestinal disorders have provided some intriguing data on the disorder. In line with this, some small studies have reported on the gut microbiome of IBS cohorts, many of which were confirmed to suffer from concomitant SIBO. One such study investigated faecal microbiota of a cohort of 30 Chinese patients with diarrhoea-predominant IBS, 14 of whom were also confirmed to have SIBO by LHBT [154]. At baseline, this cohort displayed reduced microbiota diversity, increased relative abundances of *Bacteroidetes*, and decreased relative abundances of *Firmicutes* when compared to healthy controls. In addition, the IBS cohort was shown to have reduced relative abundances of the genus *Lactobacillus*, as well as several genera associated with butyrate production. Following a 2-week course of rifaximin clinical symptoms improved and repeat LHBT demonstrated remission of SIBO in 65% of SIBO-positive subjects. However, faecal microbiota displayed only minor alterations in taxa post-treatment, a result which has been mirrored in similarly designed trials of rifaximin in IBS [155, 156]. Indeed, it must be reiterated that less than half of the IBS cohort studied in this trial were confirmed to have concomitant SIBO and the samples investigated (i.e. faeces) may bear little resemblance to the site most relevant to the disease; therefore, no definitive conclusions on SIBO pathogenesis should be drawn from this data.

With regard to SIBO in children, one study outlined above aimed to investigate microbiota in sub-Saharan children with stunted growth [6]. In addition to faecal microbiota analysis, it was deemed pertinent to retrieve and analyse microbiota of small intestinal aspirates, due to its role in nutrient absorption and malnutrition. The authors described a form of microbial "decompartmentalisation", in which oropharyngeal-associated microbes were found to be over-represented in the small intestine of these children, 91% of whom tested positive for SIBO. Although this was deduced primarily from culture-based methods, it is consistent with preliminary reports suggesting that SIBO is not caused by migratory colonic microbes [157]. In turn, 16S rRNA sequencing of duodenal microbiota in these subjects revealed a community containing near-equal parts *Proteobacteria* (32.4%), *Bacteroidetes* (29.6%), and *Firmicutes* (25.6%), with lower numbers of *Fusobacteria* (9.2%) and *Actinobacteria* (1.7%). Critically, however, this study was severely limited by the fact that there were no appropriate controls. Therefore, we are left, once again, with a degree of speculation on the potential role and composition of the small intestinal microbiota in SIBO.

These studies demonstrate that SIBO has been regarded largely as a sign or sequela of a gastrointestinal disorder, rather than a discrete disorder in itself. This perspective has meant that the pathogenesis of SIBO has been rarely addressed directly, but rather has been of peripheral interest in studies where there has been an overlapping interest. Having said this, perhaps the most informative effort to characterise SIBO to date has come from a recent investigation of 126 adults displaying gastrointestinal symptoms (66 SIBO positive and 60 SIBO negative) [158]. The authors found that, although duodenal aspirate culture results did not correlate with symptoms, the aspirate microbiome was significantly altered in symptomatic participants. This altered microbiome was characterised primarily by decreased levels of the genus *Prevotella* and enhanced microbial metabolism of ascorbic acid. In addition, it was identified that age, recent antibiotic exposure, PPI use, and diet were the major contributors to the disruption of the microbiome and onset of symptoms. In line with this, the investigators demonstrated that the restriction of dietary fibre in healthy previously high-fibre consuming individuals had significant effects on the microbiome and triggered gastrointestinal symptoms regarded as common in SIBO. This study identified several potentially targetable components of SIBO pathogenesis and

represents an excellent blueprint for the future study of the disorder.

Treatment

The first goal in the management of SIBO should be to identify and address, where possible, any underlying causes, be they anatomical (such as stenoses or fistulae), pathological (e.g. celiac or inflammatory bowel disease), or physiological (e.g. suppressed acid secretion or impaired motility). In many cases, unfortunately, either the underlying cause cannot be corrected (e.g. short bowel) or none is apparent and management rests on the suppression or elimination of bacterial overgrowth. Nutritional deficiencies related to SIBO must also be addressed.

Antibiotics continue to represent the mainstay of SIBO management. Due to the relative inaccessibility of duodenal samples for culture and difficulty in differentiating the culpable microorganism in a diverse ecosystem such as the small intestine, antibiotic therapy is generally initiated on an empiric basis. A previous meta-analysis of antibiotic use in the context of SIBO found that the non-systemic antibiotic, rifaximin, was by far the most commonly used [159]. While the meta-analysis ruled in favour of antibiotic use over placebo (effectiveness ratio 2.55, CI 1.29–5.04), rifaximin failed to reach a significant degree of superiority (effectiveness ratio 1.97, CI 0.93–4.17). However, just three studies were deemed appropriate for this analysis and their heterogeneity limited the applicability of this result. In line with this, the systematic review went on to reveal that monotherapy with 1200 mg/day rifaximin was efficacious, with an overall 60.8% remission rate. Moreover, the efficacy of this antibiotic was increased to 85% when combined with partially hydrolysed guar gum, albeit in a single trial. Two studies included in the systematic review investigated the use of metronidazole, demonstrating a normalisation of breath tests in 51% of SIBO patients, while a single small study reported remission in all 14 patients recruited and treated with ciprofloxacin.

A more recent systematic review and meta-analysis of rifaximin therapy in SIBO included data from over 1300 patients [160]. A dose-dependent response was demonstrated for eradication rates, and, in line with the previous systematic review, the most commonly used dose was 1200 mg/day, with one study reporting 600 mg/day and another 1600 mg/day. The investigators found an overall eradication rate of 70%, with adverse events reported in less than 5%. In addition, in a subset of studies which assessed symptom severity and resolution, a meta-analysis revealed symptom remission in 68% of patients who were found to have been successfully eradicated.

While the studies included in the above meta-analysis were all conducted on adult cohorts, one study previously investigated the use of rifaximin in children [161]. Applying a regimen of 600 mg/day for 1 week, the investigators reported a breath test normalisation rate of 64% (n = 21/33). In light of the limited available data, a dose response study of rifaximin in a paediatric population would indeed be of interest. Finally, one study investigated the efficacy of a combination regimen of trimethoprim-sulfamethoxazole (TMP-SMT; 30 mg kg^{1} d^{1}) and metronidazole (MTZ; 20 mg kg^{1} d^{1}) twice daily for 2 weeks in slum-dwelling children suffering from SIBO [162]. When retested for SIBO by breath test 1 month after commencement of this therapy, the authors noted 95% (n = 19/20) resolution of the disorder. However, the lack of a placebo or non-intervention control group limits assessment of temporal effects on SIBO status. Taken together, these results indicate that antibiotics are an effective means of treating SIBO in children; the paucity of studies overall and of comparative studies, in particular, limits our ability to make definitive recommendations.

While antibiotics remain the first line and gold-standard approach to SIBO management, there are additional or alternative approaches that may have applications in the future, but the efficacy of which remains uncertain at present. For instance, there is biologic plausibility to the hypothesis that there may be a role for a low-FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) diet in decreasing fermentable substrates in the context of SIBO. Low-FODMAP diets aim to greatly deplete or entirely eliminate the highly fermentable simple carbohydrates which are commonly found in certain dairy products, fruits, vegetables, nuts, and legumes, with the ultimate aim of graded reintroduction of specific FODMAPs to elucidate the most culpable source [163].

Interestingly, probiotics have also previously been considered as potential agents in the management of SIBO [164]. In line with this, a more recent meta-analysis revealed that probiotic interventions resulted in reduced H_2 levels and increased rates of decontamination when compared to placebo. More intriguing, however, is that probiotics also demonstrated more favourable results when compared directly to metronidazole treatment. Despite this, the best results overall were obtained when probiotics were combined with rifaximin or minocycline. Indeed, a recent open-labelled clinical trial performed in a cohort of patient with concomitant SIBO and systemic sclerosis found that *Saccharomyces boulardii* in combination with metronidazole was considerably more efficacious in resolving the condition than either therapy in isolation after 2 months of treatment [165].

Finally, there is some evidence to suggest that certain statins may have a role in depleting *Methanobrevibacter*

spp., thereby offering a novel therapy for methane-specific SIBO [166, 167]. In line with this, a modified-release formulation of lovastatin, termed SYN-010, has been created to deliver the drug in a biphasic manner during transit, thereby avoiding considerable degradation and absorption in the upper gastrointestinal tract [168]. A dose of 42 mg/day of SYN-010 for as few as 7 days was demonstrated to significantly reduce methane production when compared to placebo in a multi-centre double-blind randomised controlled trial [169]. The efficacy and safety trial of this intervention is currently being investigated in IBS (ClinicalTrials.gov ID: NCT03763175). The effect of this drug appears to be primarily due to the inhibition of HMG-CoA reductase, thereby preventing the formation of mevalonate, a primary precursor of key membrane lipids specific to archaea. This mechanism, along with several others, has been previously reviewed extensively elsewhere [40] and so will not be discussed further herein.

Prognosis

At present, it is unclear whether the changes in gut microbiota that characterise paediatric SIBO have long-term detrimental effects; large-scale, longitudinal prospective studies are warranted.

Conclusion

Paediatric SIBO is a heterogenous and poorly understood entity, whose prevalence and incidence are difficult to determine due to lack of uniformity and consensus of its diagnostic criteria. However, based upon the available literature, it appears that SIBO is a common underlying diagnosis in children who present clinically with certain functional disorders and stunted growth, as well as in children with a history of acid-suppressive therapies, intestinal motility disturbances, anatomical alterations, or impoverished conditions. Further research with integration of culture-dependent and culture-independent approaches is needed in order to (1) understand the pathogenesis of paediatric SIBO, its clinical presentation and prognosis, and (2) establish global consensus on diagnostic criteria for SIBO.

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Short Bowel Syndrome

43

Cecile Lambe and Olivier Goulet

Introduction

Short bowel syndrome is the leading cause of intestinal failure in adults and in children. By definition, SBS is due to intestinal malabsorption following the resection of a significant portion of the small intestine. Intestinal failure (IF) can be defined as a critical reduction of the gut mass or its function below the minimum needed to absorb nutrients and fluids required for adequate growth in children [1].

IF due to SBS may be reversible or irreversible, depending on a number of factors such as the underlying cause of SBS, the length of the remaining intestine, the preservation of the colon, the ileocecal valve, and the treatment used to develop or restore intestinal capacity.

Definition and Etiology

SBS may be secondary to prenatal diseases causing abnormal development of the GI tract (intestinal atresia, gastroschisis, extensive aganglionosis, etc.) or post-natal ischemia-related events such as midgut volvulus, necrotizing enterocolitis (NEC), trauma, and tumor. SBS remains a rare disease but its prevalence increases due to better management and overall survival. However, the true incidence of SBS is not precisely known since its early management involves both pediatric surgeons and neonatologists. The anatomy of the residual intestine is essential to predict gut adaptation and to guide management. The degree of malabsorption is proportional to the length of jejunum resected and will be compensated to some extent by the ileum and/or by the process of adaptation in response to the loss of intestinal surface. The colon is also an important player in reducing diarrhea and malabsorption.

SBS usually follows extensive surgical resection leaving the SB length below a critical value for adequate nutritional supply. A severely reduced mucosal surface results in malabsorption with subsequent diarrhea, water-electrolyte imbalance, and malnutrition [1, 2]. Exact measurement of the remnant intestine remains difficult even with the help of radiographic assessment [3]. At birth, term neonates have a SB length of approximately 250 cm and their intestines lengthen during the first year of life [4]. Preterm infants have a greater potential for bowel growth since their intestines lengthen substantially during the last trimester of gestation [5]. "Short" bowel is difficult to define clearly in children since the length of the small bowel changes so much according to the term of birth: a measure from the ligament of Treitz at first surgery, a measure compared to the birth term, a percentage of resection, etc. Befza et al. [6] have suggested using the percentage of expected small bowel/large bowel length for age based on established norms. It is an interesting approach but unfortunately it is difficult to apply in clinical settings. Our advice would be to quote the measure of the remaining small bowel length from the ligament of Treitz or from the pylorus and to note at what age the measure was performed, usually at first surgery. The cutoff length for SBS is related to a number of factors. In general, SBS occurs after a massive resection leaving less than 40 cm of viable small bowel. A residual bowel length of only 15–40 cm has been associated with bowel adaptation, intestinal autonomy, and PN weaning, but, most of the time there is scant information regarding long-term growth [6–14]. In adults the cutoff length is 150 cm. In children, patients should be considered with SBS if the remaining short bowel length is inferior to 80 cm but this cutoff can vary according to the age of the child (the older the child, the higher the cutoff length). Numerous factors determine SBS prognosis: the underlying diagnosis, the type of segments preserved, the presence of the ileocecal valve (ICV) and the colon, a long-term stoma

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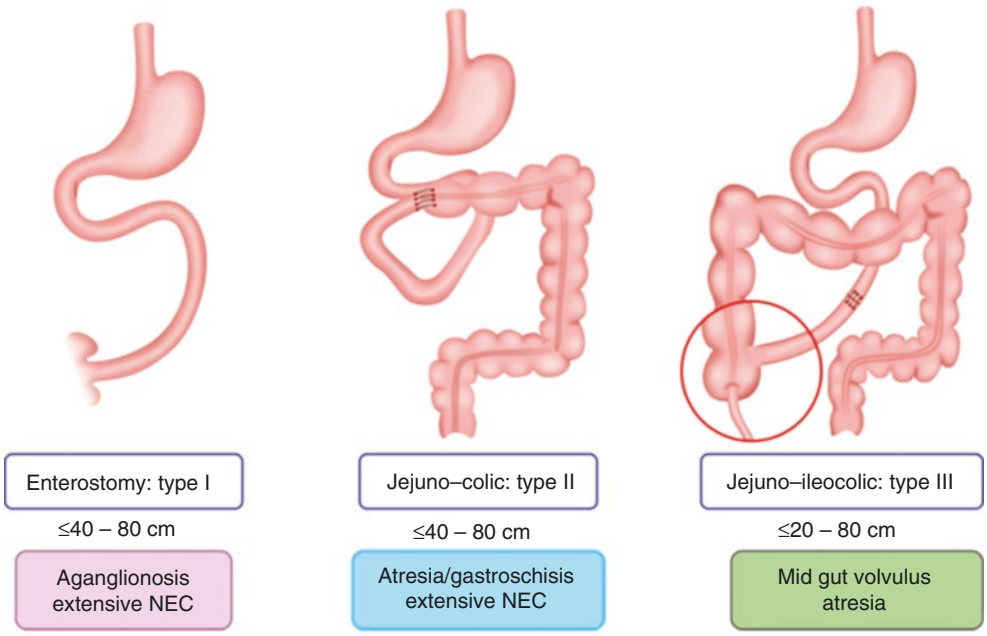

Fig. 43.1 Anatomy of short bowel according to the type of the remnant bowel. The most frequent cause of resection is mentioned for each type

versus a primary anastomosis, the number of surgical procedures, as well as the age of the patient at the time of surgery [6–14]. Classification of SBS in three types is helpful for the understanding of different outcomes (Fig. 43.1). Other factors are relevant to the development of SBS such as the functionality of the residual bowel, especially the motility disorders [15].

Intestinal Atresia

Intestinal atresia is the most frequent congenital abnormality causing SBS. The classification of jejunoileal atresia delineates four different types from a mucosal defect with an intact mesentery to multiple atresia with V-shaped mesenteric defects. Type IIIb, also called "apple peel syndrome," is the most common with a proximal jejunal atresia with a distal bowel that develops around a single retrograde blood vessel. Multiple intestinal atresia has been associated with severe combined immune deficiency [16–18] (Fig. 43.2).

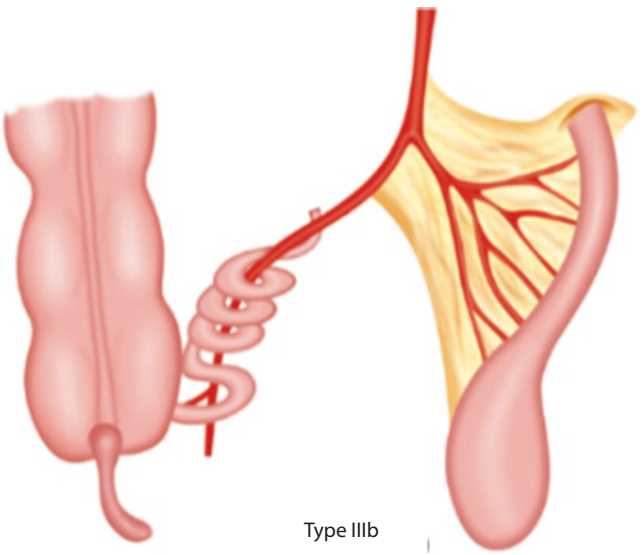

Fig. 43.2 Intestinal atresia: apple peel syndrome

At birth there are signs of bowel obstruction. Surgery procedure with end-to-end anastomosis or jejunostomy, with or without tapering, should be performed early and is highly dependent on the extent of the atresia, the importance of the distension of the proximal small bowel, and the experience of the surgeon. Motility disorders are common on both segments but can recover with time and appropriate care [15].

Gastroschisis

Gastroschisis is an abdominal wall defect characterized by an intact umbilical cord with evisceration of bowel generally to the right of the cord and without a covering membrane. Incidence in Europe is about 3 per 100.000 births [19, 20] and is due to unknown factors although maternal age, drugs during pregnancy, and genetic factors have been identified to play a role [21]. Around 40% of babies with gastroschisis are born to a mother under the age of 20 compared with only 9% of overall births. Prenatal diagnosis is routinely performed allowing choice of the place, the timing, and the mode of delivery. Most clinicians agree on the need for a C-section when the liver is outside the abdomen; in other cases the mode of delivery and the place of the procedure (on the ward or in the operating room) do not seem to influence the prognosis [22, 23]. Early surgical management aims to reduce meticulously the herniated viscera into the abdomen while avoiding high intra-abdominal pressure. When immediate

reduction is not possible, the surgeon uses a prosthetic silo created from silastic or Teflon for protecting the bowel until it slowly reintegrates into the abdomen. Long-term survival of neonates with gastroschisis is over 95% [24]. However, associated factors such as intestinal atresia, perforation, ischemia, and dysmotility caused by amniotic fluid peritonitis lead to short bowel syndrome and long-term PN is necessary in 10–20% of cases.

Long-Segment Hirschsprung's Disease – Intestinal Aganglionosis

Hirschsprung's disease (HD) derives from a congenital malformation of the enteric nervous system (ENS) [25, 26]. It displays an incidence of 1 in 5000 live births with a 4:1 male-to-female sex ratio. This severe congenital condition is caused by the absence of colonic neural ganglia and thus lack of intrinsic innervation of the colon due in turn to improper colonization of the developing intestines by ENS progenitor cells. The disease has been linked to mutations in genes that encode the crucial signals for the development of the enteric nervous system, including the RET and EDNRB signaling pathways [27]. The genetics of HD is complex and can involve mutations in multiple genes. In 80% of infants, the aganglionosis is confined to the rectum and sigmoid, but it may extend to encompass the entire colon (total colonic aganglionosis) or very rarely (less than 1% of HD) affect the entire intestine subtotal or total intestinal aganglionosis (TIA).

The TIA or sub-TIA is revealed early after birth with abdominal distention and bilious vomiting most of the time after delayed meconium passing. During surgery the extent of the aganglionic segment of the small intestine and the appropriate placing of a stoma should be guided by perioperative histopathological examinations of biopsies. Because of the absence of functional colon (SBS type 1, Fig. 43.1), infants with <80 cm of ganglionic small intestine are likely to have permanent IF requiring very long-term PN [28]. An early referral to an expert intestinal failure unit should be made for the child with TIA to optimize the fluid-electrolyte and nutritional management [29].

Malrotation and Midgut Volvulus

Midgut volvulus (MGV) can happen at any time in life. Any physician should know how to recognize the symptoms in a child with the sudden occurrence of acute abdominal pain, intermittent green bile stained vomiting, abdominal distension, and in some cases blood in the stool suggesting intestinal obstruction from the volvulus. Whether hemodynamic disorders are present or not, MGV is a surgical emergency to avoid massive small bowel infarction, and no investigations such as mesenteric vessel Doppler or contrast serie should delay the surgery. MGV in the newborn may develop as a result of malrotation but may also occur without, more often in premature infants with lax tissue. MGV may occur later in life as a result of malrotation or as a complication of previous surgery. The incidence of malrotation in children 1–18 years of age is 5.3 per million population [30]. Malrotation is caused by an abnormal process occurring during the fifth gestational week when the fetal small intestine forms a loop which extrudes into the umbilical cord. The fetal gut subsequently undergoes enlargement, elongation, and return to the abdominal cavity with rotation and fixation [31]. The fixation of the intestine may be abnormal, with fibrous bands (Ladd's bands) forming between the duodenum and the right colon with a short mesentery allowing the bowel to twist causing bowel obstruction and loss of blood supply. Intrauterine MGV is thought to be the cause of intestinal atresia type IIIb described above. Surgical management of MGV is an emergency involving detorsion of the volvulus, restoration of circulation, separation of adhesions between bowel loops, and an attempt to preserve bowel length. A conservative resection of only clearly necrotic tissue is recommended, while bowels with questionable viability should be left in situ and re-evaluated 24–36 hours later during a "second look laparotomy."

Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) remains the leading cause of SBS in premature infants. The incidence of NEC is inversely correlated with gestational age and birth weight. Incidence of NEC varies in areas around the world [32, 33]. NEC presents usually with variable symptoms: feed intolerance, gastric aspirates, abdominal distension, bilious vomiting, and blood in the stool. Local tenderness or an abdominal mass may be apparent on clinical examination and progression to pneumoperitoneum and sepsis with respiratory failure and shock. Emergency treatment of NEC includes stopping feeds supported by TPN, nasogastric decompression, fluid and electrolyte resuscitation, broad-spectrum antibiotics, and, in selected cases, respiratory support. Severity, whether mild or critical, determines the duration of medical therapy. Surgery for NEC should be limited as it may lead to bowel resection. However, surgery is indicated by (i) the presence of pneumoperitoneum, indicating perforation of the intestine, (ii) a clinical deterioration despite maximal medical treatment, (iii) abdominal mass with persistent intestinal obstruction or sepsis, and (iv) development of intestinal stricture. Acute surgical management of NEC involves competing priorities of controlling sepsis and preserving bowel length. Bowel-preserving strategies for NEC, designed to limit SBS, are based on peritoneal drainage, venting ostomy, limited resection, or a combination of them. Drainage-based strategies are generally preferred in smaller neonates, while laparotomy-based strategies are favored in larger patients, especially those with a more limited extent of intestinal injury. Comparisons of drainage-based and resection-based

approaches are limited by confounding variables, and neither approach is clearly superior with regard to subsequent SBS [34, 35]. Prevention of NEC is debatable, especially regarding the date and type of feeding [36]. Finally, the incidence of NEC has not changed significantly despite the dramatic advances in perinatal care. Given the role of inflammatory mediators in its pathogenesis, newer immune modulators are being studied as potential agents for prevention/treatment of NEC [37]. Recent data suggest that probiotics administration might be helpful in decreasing incidence of NEC in preterm infants [38].

Consequences of Gut Resection

Intestinal Resection

The more extensive the resection of the small intestine, the greater the loss of absorptive surface area for transport of nutrients, water, and electrolytes. The remaining bowel is characterized by a compromised absorptive capacity due to the severely reduced mucosal surface resulting in diarrhea, fluid and electrolyte imbalance, and failure to thrive [39]. The consequences of the resection depend not only on the length, the surface area, and the site of the resected intestine but also on the functional capacity of the remaining intestine and its ability for adaptation [6, 11, 40–43]. The first phase following small bowel resection is associated with massive losses of water and electrolyte. Severe diarrhea due to fluid and electrolyte malabsorption is increased by gastric hypersecretion, bile salts, the loss of the ileocecal valve, and undigested nutrients causing osmotic diarrhea. During this period, total PN is required in association with small amounts of substrates provided by the enteral route, orally, or by continuous gastric infusion as soon as intestinal transit has recovered.

Resection of the Jejunum

The resulting malabsorption concerns all nutrients as well as minerals, electrolytes, trace elements, and most vitamins. Severe diarrhea following extensive jejunal resection is associated with steatorrhea and creatorrhea. Moreover, the resection of the jejunum reduces the secretion of cholecystokinin and secretin leading to a decrease in biliary and exocrine pancreatic secretions which may lead, in turn, to a reduction in nutrient absorptive capacity. The reduced production of cholecystokinin, vasoactive intestinal peptide (VIP), gastric inhibitory peptide, and serotonin may cause gastric hypersecretion seen more frequently after jejunal resection. The degree of malabsorption is proportional to the length of jejunum resected and will be compensated, to some extent, by the ileum and/or by the process of adaptation in response to loss of intestinal surface. In general, resections of the proximal intestine are more manageable than distal resections because the extent of structural and functional adaption exhibited by the ileum is greater than that of the duodenum or jejunum.

Resection of the Ileum

Despite the fact that, normally, most nutrients are absorbed in the proximal jejunum, the residual ileum is able to adapt and to assume the role of macronutrient absorption. However, the specialized cells of the terminal ileum, where vitamin B12 /intrinsic factor receptors are located and where bile salts are reabsorbed, cannot be replaced by jejunal hypertrophy. Thus, the ileum has specific functions which the jejunum cannot substitute. In addition resection of the distal ileum usually includes the ileocecal valve (ICV). Ileal resection impairs vitamin B12 absorption which can cause macrocytic anemia and neuropathy. Finally, extensive loss of the ileum reduces transit time by suppressing the so-called ileal brake. It has been shown that the ileum has a greater potential for adaptation than the jejunum. In addition, ileum is the site of enteric hormone release, such as enteroglucagon, which are essential in the process of adaptation after extensive resection (see below).

Ileocecal Valve Resection

The resection of the ileocecal valve (ICV) decreases transit time (ileal brake) and allows colonic bacteria to enter and populate the small intestine increasing the likelihood of the development of a severe form of SBS [44–46]. Bacterial overgrowth may negatively impact on digestion and nutrient absorption, as bacteria compete for nutrients with enterocytes. Thus, ICV resection represents an additional major cause of malabsorption of nutrients, water and electrolytes, dehydroxylation of bile salts, mucosal injury, and motility disorders. The lack of ICV appears to greatly influence the period required to achieve intestinal autonomy following efficient small bowel adaptation [11, 40–43]. In addition, ICV resection increases the risk of sepsis of intestinal origin that occurs more frequently in infants without the ICV than in those with an intact cecum [47].

Resection of the Colon

The colon is a crucial partner for small intestinal adaptation and function in SBS patients by involving fluid and electrolyte absorption, absorption of medium-chain triglycerides, and production of short-chain fatty acids for malabsorbed energy salvage [44–46]. The removal of both ileum and colon obviously drastically increases fluid loss, dehydration, and volume depletion and causes hypocalcemia and hypomagnesemia. Preservation of the colon in cases of major small intestinal resection appears to lessen the severity of SBS.

Besides the anatomical impairment and its consequences, small bowel resection may be aggravated by several associ-

ated disorders: gastric acid hypersecretion occurs in 50% of pediatric patients with SBS, while hypergastrinemia is inconstant [48–50]. Acid hypersecretion occurs early after resection and depends on the extent of the resection. Hypersecretion is transitory but it increases with enteral feeding leading to a larger amount of intestinal fluid loss. Gastric acid hypersecretion, by reducing duodenal pH, decreases the activity of pancreatic enzymes, such as amylase and lipase, which in turn increases fat malabsorption. Gastric emptying of liquids is more rapid following jejunal resection although intestinal transit may still remain normal because of the braking effect of the ileum [50]. The loss of inhibition on gastric emptying and intestinal transit in children without a colon is related to a significant decrease in peptide YY (PYY), glucagon-like peptide I (GLP-I), and neurotensin [51]. Rapid gastric emptying may contribute to fluid losses in children with SBS.

Intestinal Adaptation After Extensive Resection

Adaptation of the remaining intestine is an extraordinary physiological process that begins soon after the resection. It is a slow process that gradually increases the bowel absorption capacity of nutrients, electrolytes, fluid, and trace elements. This adaptation process relies on the hyperplasia of the intestinal mucosa (Fig. 43.3) and the hypertrophy of the bowel in length and in width: increased diameter and wall thickness [52]. Mucosal hyperplasia is characterized by an increased number of enterocytes per unit of SB length, an increased rate of enterocyte proliferation, and an increased villous height and crypt depth. In animals, it was shown that epithelial hyperplasia following gut resection resulted in increased mucosal mass, including higher mucosal wet weight, higher protein content, as well as higher DNA and RNA content per unit of bowel length. The complex regulation of gut mucosal growth involves a multitude of factors, including hormonal mediators, such as glucagon-like peptides, neurotensin, peptide YY, growth hormone, and insulin-like growth factor [52]. Additionally, oral or enteral feeding stimulates the release of enterotrophic hormones which may further improve the process of gut adaptation [53–56]. Besides nutrients, other factors seem to play an important role in the mucosal adaptation process: epidermal growth factor (EGF) present in human milk and produced in the salivary glands and duodenal cells is a trophic substance for the gastrointestinal tract [57, 58]. Polyamines (spermine, spermidine), whose synthesis is dependent on ornithine decarboxylase activity, greatly enhance intestinal cell turnover and protein synthesis [59]. Exogenous prostaglandin has been shown to stimulate mucosal hyperplasia in the gastric antrum and in the jejunum [60].

Role of the Colon in the Adaptation Process

When preserved, the colon, by hosting the largest part of intestinal microbiota, plays a predominant role in the physiological adaptation of the intestine after intestinal resection. The colon is capable of reducing loss of energy and producing trophic factors [45]. In animal models, supplementation of an elemental diet with pectin, which is fermented to short-chain fatty acids (SCFAs) in the colon, improved adaptation of the small intestine and colon in SBS [61]. The supplementation of parenteral nutrition with SCFAs or their intra-cecal infusion reduced mucosal atrophy and intestinal immune dysfunction following massive small bowel resection [62].

In addition to their local effects, systemic SCFAs, in animal studies, can affect the motility of both the stomach and the ileum through neuroendocrine mechanisms, probably through the expression of enteroglucagon family peptides

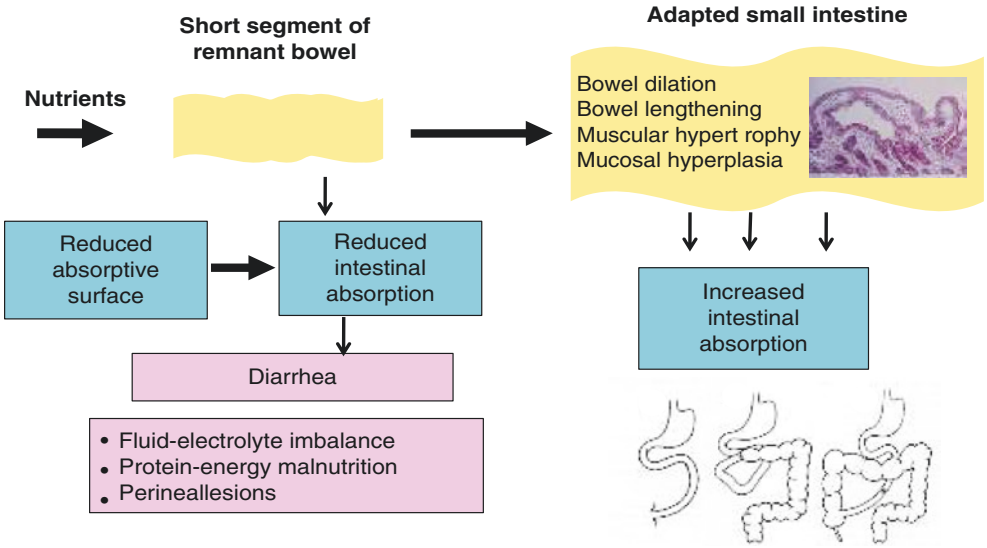

Fig. 43.3 Consequences of small intestinal resection and mechanisms of bowel adaptation

and peptide YY [63]. Furthermore, both systemic and enteral SCFAs exert a trophic effect on the jejunum by increasing mucosal mass, DNA, and villus height [64]. Since SCFAs are the preferred energy source for colonocytes, in patients with SBS the colon becomes an important organ for calorie salvage. Unabsorbed carbohydrates are metabolized by the intestinal microbiota to SCFAs [45]. In turn, SCFAs may be considered as systemic nutrients and as trophic factors directly by developing colon mucosa trophicity [65] or indirectly by promoting the release of GLP-2 [63].

Restoration of intestinal continuity, such as anastomosis of the small intestine with the colon, should be done whenever possible. With improved colonic water and electrolyte absorption, PN can then be discontinued or at least decreased. In addition, anastomosis enables colonic fermentation of unabsorbed carbohydrates from the small intestine to occur, being an important source of energy assimilation. In spite of small intestine malabsorption in patients with SBS, both hyperphagia and adaptation of the remaining colon improve patient outcomes. A study evaluated morphology, proliferation status, and transporters' expression level in the epithelium of the remaining colon of SBS adult patients compared to controls [66]. It seems that in hyperphagic SBS patients with severe malabsorption, adaptive colonic changes include an increased absorptive surface with an unchanged proliferative/apoptotic ratio and well-preserved absorption NHE2, NHE3, and PepT1 transporter mRNA levels [66]. As mentioned before, the preservation of the colon and its associated microbiota is essential for energy salvage, in reducing the need for PN and in improving the outcome of SBS patients. Bacteriological analysis based on culture-dependent methods has found that the microbiota of SBS patients are mainly composed of lactobacilli, but neither qualitative nor quantitative information is available regarding the other main bacterial groups [67]. Few data have been reported in pediatric SBS but have mostly shown low intestinal dysbiosis and reduced microbiota diversity [68, 69]. In the adaptation process, the colon becomes almost as important as the remaining small bowel [70].

Thus, the adaptation of the remaining small bowel is dependent on exogenous and endogenous factors, emphasizing the usefulness of optimal nutritional status to produce these factors using PN and the necessity of early enteral feeding after resection especially with oral feeding and human milk.

Management

Early Management

Mothers of infants with a prenatal diagnosis of one of the conditions causing SBS should be transferred before delivery to a highly trained specialized center. After birth, early management should be conducted by a multidisciplinary team including pediatric surgeons, neonatologists, and pediatric gastroenterologists trained in clinical nutrition. Ideally, a management strategy should be established by the team with the aim of performing conservative surgery and preventing complications. Procedures should be adapted to the etiology of SBS, the medical setting, and the experience of the surgeon as seen above.

The neonatal medical team needs to be highly trained in the management of long-term parenteral nutrition, fluid and electrolyte management of the ostomy output, prevention of feeding disorders, prevention of intestinal failure-related complications, and the management of central lines. ESPGHAN guidelines [71] are helpful for the management of PN but do not take into account many specificities of neonatal SBS. The end of the first phase of management is considered to be accomplished when the patient has recovered from the surgical procedure and is stable on PN with controlled intestinal losses and motility.

Long-Term Management of SBS: PN, Oral Eating, and Prevention of Complications

Once the acute phase is over, the management relies on three main areas:

- Provision of parenteral nutrition to ensure normal fluid-electrolytes status and growth.
- Promotion of bowel adaptation with oral feeding.
- Prevention of complications.

Growth with Parenteral Nutrition

Growth is essential and cannot be jeopardized. Parenteral nutrition should provide all the adequate nutritional supply to the child allowing optimal growth. Such nutritional support should follow the latest ESPGHAN recommendations [71] which include the use of 1.5–2.5 g/kg/day amino acids through a pediatric solution, a caloric intake consisting of 70–80% of nonprotein energy substrates, and 20–30% energy provided by a 20% intravenous fat emulsion.

Maintenance amounts of vitamins and trace elements are added to the PN solution by using commercially available preparations. Calcium, phosphate, and magnesium are also added to the solution according to the patient needs and the stability of the solution. Composite lipid emulsions containing fish oil have been shown to be beneficial to prevent cholestatic liver disease.

During the early phases of therapy, serum electrolytes, glucose, urea, and calcium should be measured daily. When the patient's condition and the intestinal losses become stable, blood monitoring can be less frequent. Excess fluid and electrolyte losses may complicate the management of SBS

patients, particularly in patients with high-output jejunostomies. Fluids with a sodium concentration of 130 mEq/L are used to replace jejunostomy. Replacement fluids intravenously may be adjusted based on electrolyte concentration of the lost fluids. Monitoring urine sodium concentration for excessive retention (<10 mEq/L) may indicate the need to provide more sodium, even if serum values are near normal. Excessive magnesium losses can occur with large ostomy volumes, and appropriate magnesium replacement may also improve calcium utilization. Zinc and selenium losses increase with watery diarrhea and high ostomy output. Zinc deficiency can lead to a decreased activity of zinc-dependent intestinal metalloenzymes, such as alkaline phosphatase, leucine aminopeptidase, and other intestinal disaccharidases, and delay intestinal adaptation. Zinc supplements are often used empirically, given that serum values do not reliably reflect body stores.

During the first few months after resection, H_2-receptor blocking agents (ranitidine/famotidine) should be given intravenously to inhibit gastric hypersecretion [48, 72] added to the PN solution, the drug being delivered as a continuous infusion. H_2-blockers, by increasing duodenal pH, also improve the digestion and absorption of nutrients.

Parenteral nutrition should not be stopped until normal growth can be achieved with oral and/or enteral feeding alone. Once the patient is stable, discharge on home parenteral nutrition (HPN) should be considered and organized, home parenteral nutrition being the only viable option for long-term parenteral nutrition. A common mistake is to stop PN when it is considered that the child tolerates enough enteral caloric intake according to RDA, under-estimating the residual intestinal malabsorption. The goal of intestinal adaptation is to develop the ability to withdraw artificial nutrition, thanks to a compensatory increase in the mucosal surface area and absorption capacity. The two indicators which have historically been used to predict weaning from PN in children, residual bowel length measured at final surgery and serum citrulline, though helpful, have not proven to be highly reliable prognostic factors in SBS patients [73–76]. In clinical practice, the degree of intestinal insufficiency may be measured by the percentage of PN required for normal body weight gain and growth. PN requirements remain, therefore, the best measure of the degree of intestinal sufficiency in this setting [2].

Indeed, progress in intestinal adaptation should be assessed using the *decrease of PN intake* instead of the *increase of enteral intake* which is widely used in publications. We do point out this change of paradigm to take into account the malabsorption syndrome inherent to SBS. Expressing PN dependency in nonprotein calories divided by REE (calculated using Schofield equation) allows us to have an indirect marker of intestinal adaptation according to the age, the gender, the weight, and the height of the child [77]: if the ratio decreases, the PN dependency diminishes reflecting the increase of intestinal absorption capacity, independently of enteral intake. The gold standard for measuring intestinal absorption is a stool balance analysis consisting of the collection and the measurement of all food intake and all fecal output by bomb calorimetry over a period of 3 days, but cannot be conducted in clinical settings especially in small children [76, 78].

Promotion of Bowel Adaptation

The management of SBS patients aims at promoting the physiological process of bowel adaptation by using as much as possible the GI tract especially by oral feeding (OF) which is more physiological than enteral tube feeding (ETF) [79]. PN itself aims in promoting normal somatic growth during the time bridge for achieving full intestinal autonomy. PN should not be stopped until adequate growth can be achieved with only OF and/or ETF.

The optimal strategy for enteral feeding, OF versus ETF and bolus versus continuous, remains debatable [79]. As seen above, the secretion of factors contributing to intestinal adaptation is related to the arrival of undigested nutrients in the intestine. Moreover, OF promotes the release of epidermal growth factor (EGF) from salivary glands and increases GI secretion of trophic factors [80]. Sialoadenectomy in animals significantly attenuates villous height, total protein, and DNA content after small bowel resection that is reversed by the administration of both systemic and oral EGF [81]. The advantages of oral feeding, when feasible, include the maintenance of sucking and swallowing functions, the learning of hunger-satiety mechanisms, nutritional self-regulation, the interest and enjoyment associated with eating, as well as the stimulation of growth factors and hormones released by the GI tract promoting adaptation. Alternating fasting and feeding periods along with cyclical PN avoids permanent secretion of insulin and excessive fat synthesis and deposition (steatosis, fat body mass).

Feeding must be started as soon as possible after surgery. In an infant, breastfeeding should be encouraged, or when it is not possible, an extensively hydrolyzed formula with a high proportion of medium-chain triglycerides can be used [79–82]. Breast milk contains IgA, growth factors, nucleotides, long-chain fatty acids, glutamine, and other amino acids that promote intestinal adaptation [83–87]. In addition, breastfeeding has been associated with improved immune function, as well as the genesis of a fecal microbiota rich in lactobacilli and bifidobacteria, all of which may improve SBS prognosis. On the other hand, breast milk contains lactose, which is sometimes not well tolerated in patients with reduced intestinal surface area. Human milk also contains glutamine and growth factors, such as epidermal growth factor, which possibly promote bowel adaptation. Extensively hydrolyzed formulas are mostly used when whole protein feeds are not tolerated, while amino acid-based formulas (AABF) have been used as a last resort. The AABF are generally used in the treatment of food allergies or in case of

milk protein hydrolysate intolerance [88, 89]. True food allergies have been rarely documented in children with SBS. Two retrospective studies report that the use of an AABF was associated with earlier weaning from PN and also a reduced rate of allergies [90, 91]. However, the very small sample sizes and the lack of a control group in these studies limit the applicability of these findings and do not support the recommendation of using AABF to all children with SBS and IF. Moreover, commercially available AABF contain lower levels of MCT than EHF. Finally, if the child has very short bowel with food aversion, a more palatable formula such as a classic polymeric formula with lactose can be used to stimulate oral feeding.

When oral feeding is not possible, ETF may be administered either continuously or in frequent small boluses, initially aiming at supplying a minimal enteral feeding (20–25 Kcal/kg/ day) and then increased gradually as tolerated with larger boluses during the day and continuous feeding overnight. In addition, aggressive ETF may also result in an overloaded gut syndrome with abdominal discomfort, intestinal distension, and loss of self-regulation of intake leading to eating disorders.

Feeds should be increased gradually as tolerated. Too much may be deleterious. Tolerance is key and should be evaluated by measuring stool number and volume and by the observation of vomiting, irritability, and intestinal distension. Many factors can affect stool volume in SBS, including the length of the residual intestinal segment and the type of segment (the more proximal the resection, the larger the fluid and sodium losses), and the mucosal and endoluminal variables (residual enzymatic activity and absorptive capacity, bacterial overgrowth). On one hand, *continuous* feeding appears to improve growth and adaptation through the optimization of absorption by challenging the enteral capacity [82, 92], but on the other hand forced continuous ETF may result in bowel dilation [93].

Thus, the mode of feeding still remains debatable among the different centers. In summary, feeding a child with SBS should always take into account the physiology, the psychological behavior of the infant (mother-child feeding relationships, acquisition of oral skills, abdominal and digestive comfort of the child), and finally the nutritional efficiency. Oral feeding should always be promoted even in small quantities. Continuous tube feeding should be avoided: despite the slight increase in absorption, the changes in motility patterns it induces (Figs. 43.4 and 43.5) lead to abdominal dilation and eventually deleterious SIBO. As seen above, enteral feeding is the most important

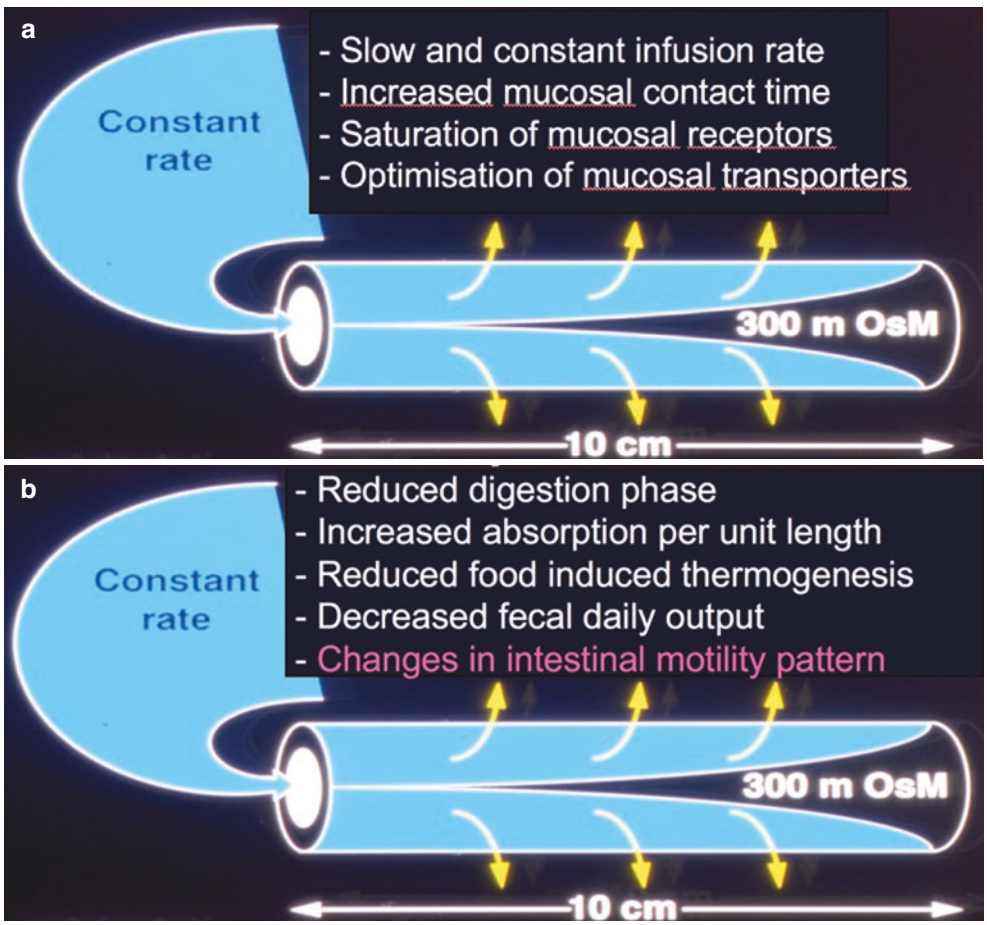

Fig. 43.4 Continuous feeding increases absorption but induces change in motility pattern

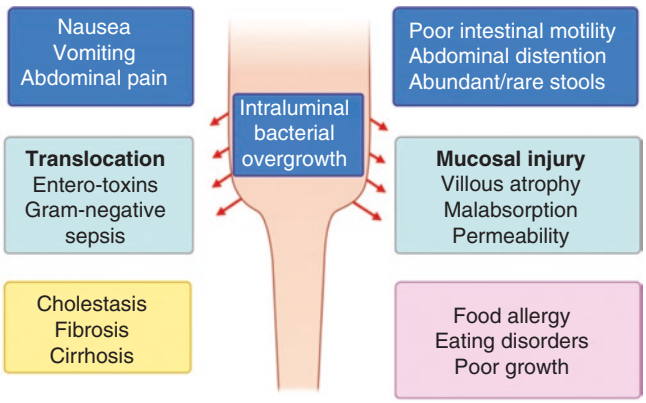

Fig. 43.5 Consequences of overfeeding a dilated intestine with subsequent intestinal stasis and small intestinal bacterial overgrowth (SIBO)

factor to promote adaptation; nevertheless, it is important to avoid overfeeding.

Prevention of Complications

Small Intestinal Bacterial Overgrowth (SIBO)

SIBO is a specific complication of SBS in patients with IF. Small intestinal bacterial overgrowth (SIBO), characterized by the presence of excessive bacteria in the small intestine, is typically described as a malabsorptive syndrome occurring in the context of gut stasis syndrome. It is generally accepted that CTF offers the advantages of optimal digestion and absorption rate [82, 92]. However, continuous infusion changes the intestinal motility pattern in the absence of a fasting period [93]. Significant dysmotility – impairing intestinal bacterial clearance – leads to small intestinal bacterial overgrowth (SIBO) with subsequent translocation and Gram-negative sepsis. SIBO and cholestasis are common especially in patients without ICV and those having abnormal motility (e.g., intestinal atresia, gastroschisis, NEC). Aggressive continuous ETF is often attempted for mimicking "hyperphagia" and enhancing absorption with the aim of weaning the child off PN, which is thought to be the cause of liver injury. These patients present with dilated loops of bowel containing residual non-absorbed nutrients. This strategy results in increasing SIBO that causes mucosal inflammation and increased permeability leading to sensitization and allergy as well as bacterial translocation, sepsis, cholestasis, and irreversible liver disease [2, 93, 94] (Fig. 43.5).

Diagnosis of SIBO remains challenging. Classic clinical signs include feeding intolerance, vomiting, nausea, liquid stools – although the colon is in continuity – higher malabsorption, higher dependence to PN than expected, and liver disease. There is no easy biological tool for the diagnosis. There is hope in the application of modern molecular techniques to the study of the small intestinal microbiome, together with some innovative sampling techniques, such as real-time intestinal gas sampling, which may soon allow the clinician to truly define the spectrum of SIBO [95].

Treatment of SIBO is controversial – ranging from large spectrum antibiotics in the short or the long term, to prebiotics or probiotics [96–99]. Repeated administration of large spectrum antibiotics may improve SIBO temporarily but lead to the risk of selection of highly resistant bacteria without treating the underlying cause of SIBO. Stopping aggressive ETF and performing nontransplant surgery to reduce the small intestine diameter may restore intestinal motility and decrease SIBO (see below). Indeed, surgical procedures such as longitudinal intestinal lengthening and tailoring (LILT) or serial transverse enteroplasty (STEP) aim not only to enhance the bowel length but also to reduce the diameter of dilated intestinal loops, thus, improving its motility and reducing SIBO [100–102].

In conclusion we consider that the best treatment for SIBO is surgery, which is far more efficient and less deleterious than antibiotics. Often the same causes repeat the same effects. It should always be questioned why SIBO developed in the first place – the main factor being a dilated loop – either caused by force feeding (usually continuous tube feeding) or anastomosis stricture or dysmotility due to the cause of SBS or a combination of all three. To avoid the recurrence of dilation and SIBO, it is critical to understand the underlying mechanism and to stop force feeding.

IFALD

IFALD is due to a combination of factors, but with the recent improvements in parenteral nutrition and lipid emulsion, these days it is mainly due to SIBO in SBS-IF patients. Many academics continue to talk about *PN*-related liver disease, suggesting that PN is the cause of liver disease. Cholestatic liver disease (CLD) has been shown to be more frequent in SBS patients than in any other IF conditions [103]. Out of 175 neonates with abdominal pathology requiring surgery, the patients with SBS (n = 40) suffered significantly more morbidity than the group without SBS in all categories of investigation (surgical complications, septic events, CRS, PN weaning delay, liver disease, and duration of hospitalization). The case fatality rate was 37.5% in patients with SBS versus 13.3% in patients without SBS (P = 0.001). Most deaths were caused by liver failure or sepsis and occurred within 1 year from the date of surgery. Nowadays, the most appropriate wording should be *intestinal failure*-associated (or related) liver disease (IFALD) [2, 104, 105]. It is probably the most important and the most life-threatening complication affecting children with SBS-IF on long-term PN. The prevalence of the disorder is unknown since there is no established definition of liver disease in this setting, and it is unclear as to whether IFALD should be diagnosed on the basis of clinical, biological, or histological criteria. However,

persistent elevation of serum conjugated bilirubin level is considered the most relevant biochemical marker of progressive IFALD. The main factors contributing to liver injury in these patients are recurrent catheter-related sepsis, prematurity and low birth weight, lack of enteral feeding, disruption of entero-hepatic biliary acid cycle (proximal stoma, ileal resection), intestinal stasis, and SIBO (obstruction, dysmotility, lack of ileocecal valve, aggressive tube feeding, etc.). It should be stressed that the most important factors leading to IFALD are those related to individual patient characteristics and, importantly, the episodes of catheter-related bloodstream infections or SIBO [106–108]. Interestingly, Finnish pediatric surgeons reported an association between bowel dilation, bowel-derived sepsis, and cholestatic liver disease [94], confirming the hypothesis of a link between bowel dilation, SIBO, and IFALD in SBS patients [2]. Factors that link infection to cholestasis are either cytokines or microbial TLR2 or TLR4 agonists [109]. Liver targets primarily include hepatocytes, but also extend to Kupffer cells, cholangiocytes, endothelial cells, and stellate cells. There are no direct studies of bile flow in humans given endotoxin, but there is sufficient indirect evidence to link endotoxin and endotoxin-induced cytokines to cholestasis. During severe sepsis, including septic shock, hyperbilirubinemia is usually a central clinical finding, often out of proportion to typically mild elevations in serum transaminase. Interestingly, TNF alpha administered in humans has shown significant hyperbilirubinemia, further supporting a link between cytokines and cholestasis [110]. An important role in this process is played by liver inflammation caused by extra-hepatic infections in which microbial products brought to the liver through the bloodstream, either directly or through production of cytokines, lead to alterations of the bile flow. The inflammation associated with these changes may cause rapid fibrosis and eventually biliary cirrhosis with end-stage liver disease [106, 111–114].

IFALD develops frequently at a very early age, especially in premature infants in whom liver immaturity, frequent sepsis, and necrotizing enterocolitis (NEC) facilitate liver inflammation and severe damages. At this young age PN is most often administered continuously over 24 hours and CRS is common. The combination of these factors makes the onset of cholestatic liver disease likely. The treatment relies on preventative strategies and acting on reducing the risk factors. Ursodeoxycholic acid (UDCA) can be implemented if tolerated. Preventative strategies should focus on oral feeding to increase bile flow and intestinal bacterial clearance, the avoidance of continuous tube feeding, the prevention and surgical treatment of SIBO, the restoration of intestinal continuity, the improvement of catheter care to reduce infections, cyclic PN to reduce continuous hyperinsulinemia, and the reduction of inflammation by using fish oil-based lipid emulsion.

IFALD and Intravenous Lipid Emulsions

While the pathogenesis of IFALD is multifactorial, studies have identified a possible link between intravenous lipid emulsions (ILE) containing high dose of soybean oil and liver disease [115, 116]. Three characteristics of ILE are involved as etiologic factors in the process of liver disease: high phytosterol content [117, 118], low anti-oxidant (low amount of alpha-tocopherol) of traditional emulsion containing pure soybean oil [119, 120], and the pro-inflammatory nature of omega-6 fatty acids. Fish oil-containing lipid emulsions offer several potential advantages including decreased omega-6 and increased omega-3 PUFA concentrations, high amounts of alpha-tocopherol, and reduced phytosterol content. Studies in PN-dependent children at risk for developing IFALD have shown that fish oil-based ILE reduced the risk of cholestasis and improved biochemical measures of hepatobiliary function compared with soybean-based ILE. The infusion of pure fish oil ILE (exclusively ω-3 FAs) allowed the reduction of intake of pro-inflammatory ω-6 and phytosterols while increasing the amounts of alpha-tocopherol, a powerful antioxidant agent [105, 119, 120]. The evidence gathered on the beneficial effects of fish oil-based ILE in these patients has led to its use in clinical practice. However, different approaches have been developed in North America as compared to Europe. In North America, following the study by Gura et al. [121] and Cowles et al. [122], a pure fish oil-based ILE (Omegaven®) has been promoted as the unique ILE to be available on the US market. In Europe, as in many other countries, it has become possible to use a composite ILE containing a mixture of soybean oil (30%), coconut oil (30%), olive oil (25%), and fish oil (15%) (SMOF-lipid®). Both ILEs contain 200 mg/L of alpha-tocopherol. Some concerns have been raised on providing fish oil ILE as the sole source of lipids over a long period of time. Omegaven® provides less essential ω-6 fatty acids than what is currently recommended in infants and young children [123]. Randomized, double-blind, controlled trials in preterm babies stratified by body weight have analyzed a set of parameters (clinical data, laboratory data, fatty acids in plasma and red blood cells, plasma levels of alpha-tocopherol and alpha-phospholipids) after infusion of PN with SMOF-lipid® or soybean oil-based ILE [124–126]. The SMOF-lipid® emulsion increased the content of eicosapentaenoic EPA and docosahexaenoic (DHA) acids and reduced the ω-6/ω-3 ratio, improving also liver function tests. In a study evaluating the long-term effects of SMOF-lipid® versus a soybean oil-based ILE in pediatric patients on home PN, no differences between biochemical and nutritional outcomes were recorded, but there was a clear association between the use of SMOF-lipid® and a significant decrease of bilirubin levels that conversely increased in the soybean oil-based group [127]. Muhammed et al. examined the effect of the switch from a soybean oil-based lipid emulsion to

SMOF-lipid® in 17 children with cholestasis [128]. Over a period of 6 months, the use of SMOF-lipid® was associated with a marked statistically significant reduction in the levels of bilirubin when compared with the soybean oil-based lipid group. In a recent clinical trial, Diamanti et al. [129] showed that compared to Intralipid® (pure soybean oil-based ILE), SMOF-lipid® reduced the risk of progressive IFALD in children with intestinal failure.

Infants and children with SBS are at high risk of developing IFALD. Therefore, composite lipid emulsion containing fish oil should be preferred in these patients from the very early phase of management as a preventative measure. In the absence of available composite lipid emulsion, fish oil (Omegaven®) should be used in combination with soybean and coconut oil with added alpha-tocopherol.

Catheter-Related Issues

Infections

Central catheter-related bloodstream infections can be life-threatening, and the recurrence of sepsis is associated with cholestasis and progressive liver disease, especially in young infants [106]. Tunneled catheters should be used to prevent the migration of skin commensal flora to the bloodstream. Central catheters should always be handled with sterile technique by experienced staff, the lines should be changed every 24 hours, and the insertion site should be regularly monitored with antiseptic dressing every 72 hours.

In the case of home PN, parents and/or caregivers should be trained by specialized teams in the sterile technique of catheter handling (connection and disconnection of the PN infusion), in the insertion site dressing, and in more basic care such as giving a bath and going swimming, which, if done inappropriately, can lead to catheter infections. In case of recurrent infections or loss of vascular access, antiseptic locks (such as ethanol or taurolidine) should be implemented and allow to decrease the rate of catheter infection below 1/1000 catheter-days. Bacterial translocation from SIBO is directly linked to intestinal dilation [94] and contributes to recurrent sepsis and subsequent liver damage.

Catheter salvage strategy should always be preferred – except in case of *Staphylococcus aureus* infections – since multiple catheter insertions might repeatedly damage vein walls and promote thrombosis [130].

Exit-site infections can usually be treated using twice-daily antiseptic dressing changes and local antibiotics, but sometimes extend toward tunnelitis requiring an emergency catheter removal.

Thrombosis

Thrombosis of central venous access should be avoided at all cost since it compromises the possibility of further parenteral nutrition and may be an indication for early intestinal transplant. Thrombosis is a rare event in children but the central catheter is a major risk factor: 85% of thrombosis in children occurs in children with a central catheter. Thrombosis is caused by several factors: stasis, vascular trauma, and hypercoagulability. The catheter itself is a thrombotic surface and causes blood flow reduction due to its size in small infant veins. The parenteral nutrition solution is hyperosmotic. Premature infants have an immature coagulation system. Infections especially with *Staphylococcus aureus* increase the risk of thrombosis. Preventative measures such as inserting the thinnest catheter adapted to the size of the child, prevention and early treatment of catheter infections, and catheter salvage strategy are essential. Anti-coagulant therapy can be discussed in case of extensive thrombosis but has not demonstrated its efficacy in the prevention of catheter-related thrombosis.

Occlusion of the catheter is prevented by pulse-flushing. In case of occlusion, thrombolytic agents can be used punctually.

D Lactic Acidosis

Clinical manifestations such as abdominal distension, bloating, and nausea – due to colonic microbiological hypermetabolism – may impair daily life and should be monitored. They are the consequences of the intestinal malabsorption leading to the arrival of a huge load of undigested CHO in the colon. This condition may be worsened by hyperphagia or aggressive tube feeding. One rare complication of colonic hypermetabolism, which is clearly different from small intestinal bacterial overgrowth (SIBO), is D-lactic acidosis.

D-Lactic acidosis (D-LA), also referred to as D-lactate encephalopathy, is a rare neurologic syndrome that occurs in individuals with SBS or following jejunoileal bypass surgery [131, 132]. Fortunately, this complication is very rare. Symptoms typically present after the ingestion of high-carbohydrate feedings. Neurologic symptoms include altered mental status, slurred speech, and ataxia, with patients often appearing drunk. Onset of neurologic symptoms is accompanied by metabolic acidosis and elevation of D-lactate plasma concentration. L-Lactate concentration, which is reflected by serum lactate concentration, is normal. Thiamine deficiency should be excluded [133]. D-Lactate fecal concentration may be assessed routinely [65].

Lactobacilli and other bacteria, including *Clostridium perfringens* and *Streptococcus bovis*, ferment unabsorbed carbohydrate to D-lactic acid, which cannot be metabolized by D-lactate dehydrogenase. These organisms may proliferate in an acidic environment that may be promoted by the metabolism of unabsorbed carbohydrates to SCFAs. The mechanism for the neurological symptoms is unknown. They have been attributed to the D-lactic acid, but it is unclear if this is the cause or whether other factors are responsible [65]. Treatments described in case reports have included observation with spontaneous resolution, oral metronidazole, neomycin, van-

comycin (for 10–14 days), and avoidance of "refined" carbohydrates [134]. Probiotics, prebiotics, and synbiotics have been used but without clear efficacy [135, 136]. Prevention of D-lactic acidosis relies on avoiding high load of carbohydrates. In some patients, it might need increasing or resuming PN when symptoms are too frequent.

Perianastomotic Ulceration

Perianastomotic ulceration is a rare but severe complication after intestinal resection and anastomosis. It is described mostly in children [137, 138]. The main symptom is subclinical intestinal bleeding leading to severe iron deficiency anemia. A survey reported patients with anastomotic ulcerations after intestinal resection in infancy focusing on predictive factors, medical and surgical treatment options, and long-term outcomes [137]. Eleven patients were reported. The diagnosis was often delayed for several years. No predictive factor (including the primary disease, the length of the remnant bowel, and the loss of the ileocecal valve) could be identified. Numerous treatment options were tried, including antibiotics, probiotics, and anti-inflammatory drugs, but none proved to be effective to induce prolonged remission. Even after surgical resection, relapse was observed in 5/7 children. The mechanism leading to anastomotic ulcerations remains unknown but it is likely that intestinal microbiota dysbiosis plays an important role. Another recent study reported 14 cases revealed by severe anemia, diarrhea, abdominal pain, and growth failure in average 11.5 years after surgery [138]. Ulcerations were multiple in most cases ($n = 11$), located on the upper part of the ileocolonic anastomosis ($n = 12$), and were difficult to treat. No granulomas were seen but lymphoid follicles were frequent. In addition, either anti-saccharomyces cerevisiae antibodies (ASCA) or anti-neutrophil cytoplasmic antibodies (ANCA) were positive in 4/9 tested patients and 8/11 genotyped patients exhibited a NOD2 mutation ($P < 0.0002$ when compared to healthy controls). Contrary to previous reports with limited follow-up, no medical or surgical treatment could prevent recurrences. Because relapse may occur several years after discontinuation of PN, long-term follow-up is needed. Frequent relapses causing chronic iron deficiency anemia might benefit in the future from fecal transplantation.

Eating Disorders

Children with SBS-IF often develop oral aversions and eating disorders. It was pointed by Moreno Villares et al. that tube feeds delivered by either gastrostomy or a nasogastric tube, often used in infants to provide adequate caloric and nutrient intake, may induce eating disorders [139], along with surgery, fasting, and pain in the first weeks of life. It is important to introduce oral feeding early to avoid the development of poor feeding skills who receive artificial nutrition. Children in transition for tube to oral feeding may display oral-motor, sensory, and developmental feeding problems. There is limited research and information on which to base interventions that will preserve and develop oral motor and feeding skills. Interdisciplinary teams are needed to effectively address these complex oral feeding problems [140]. Accurate identification of the underlying issues will allow healthcare providers to develop interventions to improve feeding outcomes for children with SBS and to avoid the use of tube feeding. In clinical practice, the role of nurses for supporting the development and the psycho-motor coordination in neonates should be emphasized in order to prevent eating disorders. Later in life, it is very challenging to develop healthy eating behavior in children with long-lasting eating disorders who remain dependent on tube feeding [141].

Growth Failure

Growth failure in children with SBS is usually a direct consequence of inappropriate management and forced PN weaning. The slow transition from PN to full oral feeding requires time during which nutritional status has to be maintained at an optimal level. Trying to wean off PN too rapidly in an infant with neonatal SBS leads to the risk of failure to thrive and metabolic complications. PN intake should be increased for achieving optimal growth and nutrition. A common mistake consists in adding PN and EN calories. The sum of PN and EN even if fitting the RDA may not be sufficient for growth because of intestinal malabsorption. However, some other factors might contribute to growth difficulties in children with SBS (Table 43.1).

Other Complications

Biliary Lithiasis

Patients with SBS are at high risk of developing gallstones. The lack of stimulation of gallbladder emptying (absence of proper meals) and the disruption of the bile entero-hepatic cycle along with the use of certain antibiotics contribute to the accumulation of sludge in the gallbladder predisposing to the formation of gallstones. Premature infants are especially exposed to cholelithiasis because of their low production of conjugated bile acids.

Systematic cholecystectomy is not recommended as it can lead to surgical complications such as post-ischemic stricture of the common bile duct. Medical treatment with urso-

Table 43.1 The main causes of failure to thrive in SBS patients

Insufficient and/or unadapted parenteral nutrition intake
Trace elements deficiencies (especially zinc)
Sodium depletion from high ostomy output
Inefficient enteral tube feeding (malabsorption, SIBO)
Gut mucosal inflammation from bacterial overgrowth
Chronic metabolic acidosis (D-lactic acidosis)
Repeated sepsis (catheter related, translocation, etc.)
Intestinal failure-associated liver disease (IFALD)

deoxycholic acid is indicated in oligosymptomatic and asymptomatic lithiasis with transparent, soft, cholesterol-rich stones. Cholecystectomy is indicated in case of recurring episodes of cholecystitis or gallstone migration.

Oxalate Renal Lithiasis

In SBS patients with colon in continuity, bile salt deficiency prevents full solubilization and absorption of fatty acids, which then bind preferentially to intraluminal calcium. Under normal conditions, calcium binds to oxalate in the distal intestine, forming a precipitate that is expelled in the stool. But when calcium instead binds to fatty acids, excess free oxalate is absorbed by the colonic mucosa and excreted by the kidney, leading to hyperoxaluria and an increased risk for renal calcium oxalate stones and deposits. This complication is rare in young children but might occur in teenagers with persistent fat malabsorption and hyperphagia. To offset the risk of nephrolithiasis, patients should limit their intake of dietary oxalate and increase calcium consumption to limit residual free oxalate in the colon [142] (Fig. 43.6).

Alternative Treatments

Nontransplant Surgery

Autologous Bowel Reconstruction

Surgical approaches aiming at maximizing gastrointestinal digestive and absorptive function are crucial to the management of SBS. These include stoma closure and restoration of bowel continuity together with resection of strictures and closure of fistula. Some surgical procedures have aimed at slowing intestinal transit in the hope that it would improve absorption, such as construction of intestinal valves, reversed intestinal segments, and colon interposition: clinical benefits remain poorly defined in pediatrics. Conversely, surgical interventions aiming at increasing the length of the bowel by reducing the diameter of the dilated loop result in reducing the stasis in the dilated bowel (and decreasing SIBO in the process) and, by increasing the length of the bowel, increase the contact time between luminal nutrients and mucosa (without stasis) which might improve the overall absorption. Indeed, these procedures aim not only to increase the intestinal length but also to reduce the diameter of the dilated intestinal loop with subsequent reduction of SIBO. The most common procedures are longitudinal intestinal lengthening and tapering (LILT) developed by Bianchi in Manchester, UK [143], and serial transverse enteroplasty (STEP) developed by Kim et al. in North America [144] (Fig. 43.7a, b). The precise indications and the potential benefits of these procedures remain a matter of debate [145, 146]. Classical conditions and indications for bowel-lengthening surgery include the presence of a large intestinal diameter (>3–4 cm) for at least 20 cm of small bowel and a minimum total bowel length of 40 cm.

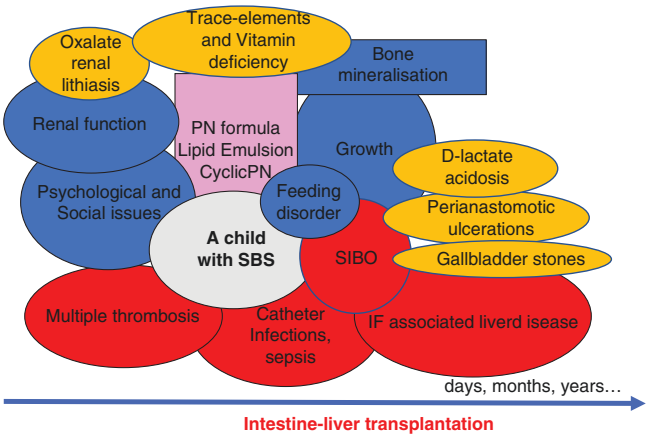

Fig. 43.6 Complications associated with SBS-IF

LILT involves longitudinal splitting of the remnant small bowel along its mesenteric and anti-mesenteric border, ending up with two tubes of bowel of identical length each with their own blood supply which are then joined together [143]. The advantages of the LILT procedure (Fig. 43.7a) include the conservation of the normal orientation of the muscular fibers allowing more physiological peristaltic contraction, and the possibility to further perform a STEP procedure on the operated segments. The disadvantages are the risk of vascular complications during surgery making LILT more technically demanding as compared to the STEP procedure [143–146]. The STEP procedure involves the use of a surgical stapler applied sequentially from alternating and opposite directions to the dilated loop, in a transverse, partially overlapping fashion creating a zigzag-like channel of approximately 2 to 2.5 cm in diameter (Fig. 43.7b). This operation has the great advantage of being simple and reproducible; unlike LILT, no anastomosis is needed, and the mesenteric blood supply is not put at risk. If the bowel re-dilates, a further STEP procedure can be undertaken. However, results after STEP and re-STEP procedure are not as performant as expected [101, 102, 147–149].

Theoretically, these two surgical techniques do not increase the small bowel surface area although they increase the bowel length. However, by reducing bowel dilation, intestinal stasis, and SIBO, they restore intestinal mucosal integrity. Indeed, a 5-year follow-up cohort study after STEP confirmed the efficiency of this procedure to increase intestinal absorption. Interestingly, both D-xylose – a marker of carbohydrate absorption and mucosal integrity – and plasma citrulline, a marker of small bowel enterocyte mass, increased significantly postoperatively [150]. This suggests that STEP procedure by reducing SIBO restores small intestinal mucosa integrity and improves villous size within the first weeks following the procedure.

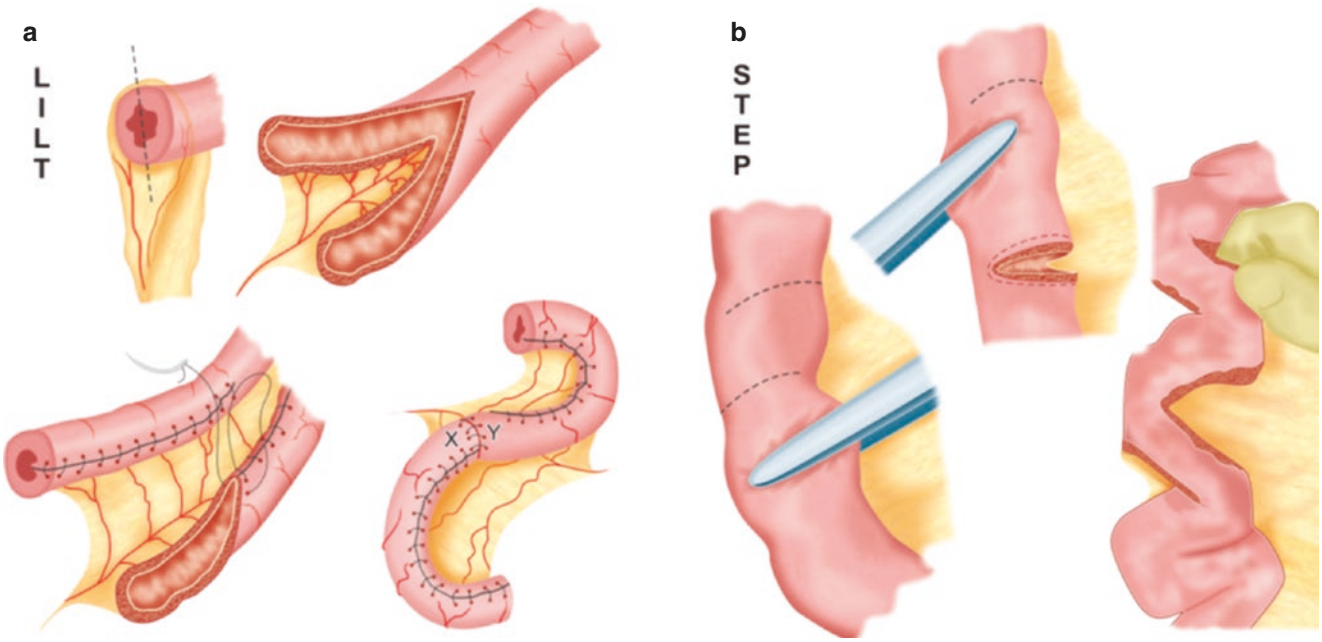

Fig. 43.7 Nontransplant surgery in the short bowel syndrome: LILT, longitudinal intestinal lengthening and tailoring; STEP, serial transverse enteroplasty

Surgical Management of TIA

The surgical management of TIA/NTIA is challenging and the role of autologous intestinal reconstructive surgery (AIRS) is controversial [150, 151]. A recent cross-sectional study concludes that AIRS may be performed in carefully selected patients [151]. It may be an effective way to enhance residual bowel absorptive function and to reduce PN requirements.

Usually, surgery is performed as a simple jejunostomy just above the aganglionic intestine at diagnosis. The subtotal enterectomy is delayed to either pull-through procedure or intestinal transplantation. In patients with TIA/NTIA, we would advise subtotal enterectomy of the aganglionic segment at an early age, to avoid chronic sepsis in the excluded bowel and its deleterious consequences on the liver. If the stoma is normally ganglionic with normal motility of the stomach, duodenum, and initial jejunum, we would consider to lengthen the small bowel by sero-myotomy (about 40 cm) and perform a new stoma in the same operation [150–152]. This procedure aims at lengthening the small bowel for about 40 cm for slowing the transit and increasing the absorption surface. A longer segment may cause obstruction since sero-myomectomy removes achalasia but does not restore motility. Our experience with this procedure is positive without obstructive symptoms. It is now clear that the aganglionic bowel left in the abdomen may cause a severe condition related to diversion colitis. The question of the loss of the abdominal domain in the view of future Tx should be balanced with the high risk of causing sepsis and liver disease in the short term. Any bacteria-related inflammation of the excluded bowel (diversion colitis), by injuring the mucosa, increases permeability, enterotoxin release, and bacterial translocation and in turn causes portal inflammation and subsequent cholestasis/fibrosis/cirrhosis. Even if these children have to undergo liver and intestinal transplantation, staged closure of the abdominal wall would be possible even with the lack of room in the abdomen. We think this does no longer justify maintaining the aganglionic bowel inside just "to keep room." Nontransplant surgery and intestinal transplantation (ITx) are complementary surgical tools in the complex management of TIA/NTIA.

New Treatments (GLP-2 Analogs, Insulin, EGF)

Hormonal Therapy and Other Adaptive Treatments

Hormonal therapy is promising in the management of infants with SBS. The role of recombinant human growth hormone (rhGH) alone or in combination with glutamine has been investigated. Inconsistent results have been reported in adults receiving rhGH, with reported side effects [153]. A few studies of rhGH alone or in combination with glutamine have been carried out in PN-dependent children with SBS [154–156]. Despite some decrease in PN requirements during treatment, these trials showed little benefit on body composition and mucosal absorption in the long term.

Epidermal growth factor (EGF) has been shown to have a role both in maintaining epithelial tissues and in controlling intestinal adaptation. In clinical setting, five SBS pediatric

patients (<25% bowel length predicted for age) received 100 mg/kg per day given mixed with enteral feeds for 6 weeks [57]. All patients showed a significant improvement in carbohydrate absorption and improved tolerance of enteral feeds (enteral energy as percent of total energy, 25% ± 28% pretreatment vs. 36% ±24% post-treatment; mean ± SD ($P < 0.05$)). EGF treatment was not associated with significant changes in intestinal permeability, the rate of weight gain, or liver function tests. However, this study involving only five patients assessed with discussable parameters did not allow to draw any conclusion whether EGF treatment was efficient and EGF treatment has not been developed any further.

Oral insulin has been shown to influence intestinal structure and absorptive function. The effects of parenteral and oral insulin on structural intestinal adaptation, cell proliferation, and apoptosis were shown in rats [157]. This favorable effect of insulin is relevant and is now considered in SBS patients [158], but is still at the stage of clinical research.

Other relevant treatments associated with a trophic effect on the bowel mucosa such as short-chain fatty acids may be beneficial in children with SBS [159].

Glucagon-like peptide 2 (GLP-2) is produced by the L-cells of the terminal ileum in response to luminal nutrients and has a trophic effect on the intestine, promoting absorption and adaptation. GLP-2 has been shown to increase the surface area of the gut mucosa, upregulate nutrient absorption, improve gut-barrier function, increase intestinal blood flow, and decrease bone resorption. The effect of GLP-2 analogs on the gastrointestinal function was assessed in patients without a terminal ileum and colon who have functional short bowel syndrome with severe malabsorption and no postprandial secretion of GLP-2 [160]. Balance studies were performed before and after treatment with teduglutide. Treatment with teduglutide (0.05 mg/kg/day) improved the intestinal absorption of fluid and energy and increased body weight. It is now demonstrated with several double-blind placebo control studies that teduglutide improves intestinal absorption and nutritional status in SBS adult patients [160, 161]. Factors associated with teduglutide response were baseline parenteral support volume, bowel anatomy, and SBS features [162]. A study was performed in young infants correlating postprandial GLP-2 levels with intestinal length, nutrient absorption, and patient outcome [163]. These results suggest that in infants with intestinal dysfunction, GLP-2 levels are correlated with residual small bowel length and nutrient absorption and may be predictive of outcome. GLP-2 might be the most logical medical approach for early management of SBS patients especially those with ileal resection. A 12-week, open-label study enrolled SBS PN-dependent patients aged 1–17 years [164]. It was concluded that teduglutide (GLP-2 analog) was well tolerated at 0.025 or 0.05 mg/kg/d and was associated with trends toward reductions in PN requirements and advancements in enteral feeding. However, study limitations included its short-term, open-label design, small sample size, and heterogeneity of both patients and management because of the multicenter study. The effects of the treatment rapidly faded at the stop of treatment to come back to pretreatment PN requirements. The study was extended to 24 weeks [165] and enrolled 59 children: 24 received 0.025 mg/kg of teduglutide, 26 received 0.05 mg/kg/day, and 9 received standard of care (SOC). The 24-week study confirmed the results observed in the 12-week study [164] with a similar safety profile and a reduction of caloric PN support of 43% in the treated groups ($p < 0.05$). Most of the reduction was observed in the first 12 weeks of treatment, and only 5 children could be totally weaned off PN: 2 of these children had a remaining small bowel length of 120 cm which hardly qualifies them as "short" bowel syndrome children and the 3 others had over 40 cm of remaining small bowel with more than 70% of remaining colon – which should have been sufficient to wean off PN without the use of teduglutide. These results question the selection of patients and raise the question of which patients should benefit from this costly treatment with a lack of hindsight for a treatment for life starting in childhood. An open-labeled monocenter pediatric study is underway using teduglutide at the dose of 0.05 mg/kg/d which included children with severe SBS-IF who had reached a plateau in adaptation aiming at evaluating the reduction of PN intake and the increase in intestinal absorption using balance studies [166]. This trial might give us more answers on which patient should benefit the treatment. Other analogs of GLP-2 (apraglutide, glepaglutide) are under trial in adult patients in order to either increase the effect on the intestine or increase the half-life of the treatment.

Inhibitors of Dipeptidyl Peptidase-4

Dipeptidyl peptidase-4 (DPP4) inhibitors are used in routine for the treatment of type 2 diabetes mellitus. Interestingly, GLP-2 analogs have been developed so they could resist longer to the action of the DPP4.

Sueyoshi et al. have experimented the use of orally active DPP4 inhibitor to determine the efficacy of this approach to promote adaptation after resection in a mouse model of SBS. Treatment led to differential effects over time with greater absorptive function at early time points and enhanced proliferation at later time points. The action seemed to peak by 30 days regarding intestinal epithelial cell proliferation and GLP-2 plasma levels [167–169]. This approach has not yet been developed for human use.

Tissue Engineering

In order to develop novel approaches to the treatment of short bowel syndrome, some research teams have focused on the development of an artificial intestine by placing intestinal

stem cells on a bioscaffold that has an absorptive surface resembling native intestine and taking advantage of neovascularization to develop a blood supply. Although there are recent advances in biomaterials, vascularization, and intestinal organoids and progress toward development of a functional epithelium and mesenchymal niche, lots of challenges still remain in the field and human application is still in the far future [170–173].

Long-Term Prognosis (PN and Weaned Patients, Growth, Adult Height)

SBS mortality decreased during the last decade following the implementation of "intestinal rehabilitation centers" [174]. Multidisciplinary management, improved prevention of SIBO and sepsis by performing autologous bowel reconstruction [143–149], prevention of catheter-related bloodstream infections with taurolidine or ethanol lock procedures [175, 176], and use of last generation of lipid emulsions [13] allowed to prevent the development of irreversible IFALD and evolution toward nutritional failure. As a matter of fact, the rate of intestinal transplantation decreased in the meantime [177].

Children with SBS who survive the first weeks of life and who are not weaned off PN in the first months of life should benefit from home parenteral nutrition with a multidisciplinary follow-up involving pediatric gastroenterologists, surgeons, anesthesiologists, dieticians, specialized nurses, psychologists, and occupational therapists. The PN formula should be tailor-made and regularly adjusted to the child's needs. About 60% of children with SBS who are discharged on home parenteral nutrition can be weaned after 2 to 4 years [76, 178]. The other patients will remain on PN, usually with one or several nights a week of PN, unless GLP-2 analog treatment allows weaning or intestinal transplantation is considered.

In patients with TIA/NTIA, appropriate management strategies are not well established. Nutritional management includes cyclic PN (home PN) associated with oral feeding for reducing the risk of liver disease and promoting oral skills. Continuous attention is needed in the daily long-term management of these unstable infants and children with a permanent risk of dehydration and subsequent complications such as hypercalcemia and renal failure. Normal growth is achieved if PN is adapted especially according to the high water-electrolyte losses.

Special consideration should be given to the link between the hospital and the home care teams. Fostering coordination of surgical, medical, and nutritional management is vital to provide high-quality, integrated care of patients with IF, thus markedly improving the survival of these patients [178]. The three most important issues in the management of children with SBS-IF include (i) a good and early link between primary caregivers and IF programs, (ii) the presence in the program of both intestinal rehabilitation and intestinal transplantation expertise, and (iii) the participation in the network of the organizations providing home PN solutions. The expertise required to prescribe PN both at home and in the hospital usually comes from a dedicated hospital-based nutritional team with a thorough knowledge of energy expenditure, nutrient and trace element requirements by age, appropriate central catheter handling, and awareness of the risk and complications of long-term PN. Home PN must be tailored to the single patient and his or her family, always maintaining the goal of counteracting the deleterious effects of intestinal failure. Official guidelines and position statements on central catheter handling and PN prescription have been published [71].

Collaborative strategies must be developed in order to reduce mortality and morbidity in patients with SBS-IF, especially for those who are referred for permanent IF or intestinal transplantation.

Some studies have reported the long-term growth and nutrition status of children with neonatal SBS after weaning off PN [22, 179–181]. Improved care of patients with SBS significantly achieved more optimal weight gain for age compared with the decade 1980 [179]. However, the final genetic target size is not always achieved, while some deficiencies may be evidenced [180, 181]. Indeed, children with SBS are still at risk for different nutrient malabsorption even after weaning off PN for a long time. They may develop such an "overloaded gut syndrome" with failure to thrive requiring, for some, PN to be restarted. Therefore, they need very long-term follow-up, regular monitoring, and intensive nutritional care to prevent various nutrient deficiencies. On the other hand, long-term follow-up is mandatory, not only for growth monitoring but for assessment of micro-nutrient deficiency (e.g.. zinc status in the case of high stool output, liposoluble vitamins, and vitamin B12 in the case of ileal resection) and search for biliary lithiasis if the gallbladder was not removed.

Nutritional Failure and Intestinal Transplantation for SBS-IF

Although a large percentage of children with SBS-IF can survive with long-term PN, a proportion of patients eventually develop life-threatening complications such as severe septic episodes, fluid and electrolyte imbalance, loss of venous access for PN, and end-stage liver disease [2, 182]. In these patients, nutrition has failed both in the enteral and the parenteral routes. These patients are considered to have "nutritional failure" and should be referred for intestinal transplantation (ITx) as early as possible [2, 182].

Unfortunately, relatively few advances have been achieved in the field of ITx and multivisceral transplantation in the last 10 years with no significant improvement in the long-term patient and graft survival [183]. According to the intestinal transplant registry, approximately 2080 ITx have been carried out in 2010 children since 1985. Overall, 1-year and 5-year patient and graft survival were 72.7%/66.1% and 57.2%/48.8%, respectively. Rejection remains the largest contributor to long-term graft loss, while sepsis is the main cause for patient death [183] and quality of life may improve [184]. These sobering figures mandate the adoption of all relevant strategies to avoid ITx until new protocols are available to achieve a better outcome.

There is probably a different threshold for ITx on both sides of the Atlantic Ocean. The European approach is more inclined to support long-term home PN, which is cost-effective and provides a better quality of life, rather than to refer a child for ITx. Support for this view comes from Pironi et al. who have performed a 3-year prospective study including both adults and children on long-term PN for IF [185]. They compared "non-candidates" for ITx (no indications nor contraindications) with "candidates" who had an indication according to the USA Medicare and Medicaid Services definitions, and a high risk of death or morbidity according to the American Society of Transplantation position paper [186, 187]. The results showed that only patients with nutritional failure due to IFALD or major catheter complications had an increased risk of death on home PN, thus supporting its use as the primary treatment for IF [187]. Therefore, it was suggested that ITx should be used only as a life-saving procedure [2, 188]. Although experienced transplantation centers have suggested that the role of ITx should be expanded to a pre-emptive/rehabilitative procedure applicable to all patients with irreversible IF, the recent findings have shown that home PN is the treatment of choice for IF in adults as well as in children. An early referral is essential to prevent or optimize the long-term management of IFALD. Central venous-catheter-related major complications might be indications for a pre-emptive intestinal transplantation in selected patients. As a matter of fact, "nutritional failure" should be regarded mostly or even only as a clear indication to ITx [2].

Isolated liver Tx has been performed for IFALD in SBS-IF patients. Taha et al. reported a group of children with SBS and IFALD who have the potential for adaptation in the residual bowel underwent isolated LTx [189]. The prognosis remains poor after this procedure – 8 survivors out of 14 [189]. This procedure should be avoided by preventing IFALD. If performed, it should be exercised with extreme caution. These children need careful assessment before isolated LTx and close follow-up with an experienced multidisciplinary team to monitor nutritional outcomes and may need consideration for transplant or nontransplant surgery in the long term [190].

In patients with TIA, ITx is undertaken according to the occurrence of complications (fluid-electrolyte disorders, sepsis, IFALD) and/or the wish of parents for another quality of life. In 12 patients with TIA, an outcome rate of 62.5% in the LITx group and 75% in the ITx group, both with half colon grafting, was reported [191]. All the surviving patients were weaned off PN, after a median of 57 days. Pull-through of the colon allograft was carried out in all patients. Fecal continence is normal in all but one of the surviving children. When long-term graft tolerance is achieved, growth is normal and quality of life improved [190, 192].

Conclusion

Pediatric short bowel syndrome requires specialized and individualized strategy from the diagnosis to the discharge home and further. Intestinal rehabilitation programs provide this complex care with the goal of achieving enteral autonomy and oral feeding without intestinal transplantation. The establishment of multidisciplinary intestinal rehabilitation programs at leading centers has improved the survival of children with SBS-IF, while the morbidity associated with both IF and PN has significantly decreased.

Recent advances in the knowledge of factors related to PN and IF complications and improvements in the medical and surgical management of SBS result in better outcomes for these patients. It is interesting to note that the most recent International Intestinal Transplantation Registry reports evidence of a worldwide trend of reduction in the number of pediatric ITx. This might be explained by at least several factors:

- Provision of guidelines and training.
- Development of intestinal rehabilitation centers with increasing IF expertise.
- Enlarged use of nontransplant surgery.
- Better prevention of IFALD, with fish oil-based lipid emulsions and SIBO treatment.
- Improved prevention of catheter-related sepsis by using taurolidine or ethanol locks.
- Promotion of oral or bolus feeds.
- Onset of hormonal treatment in SBS-IF by using GLP-2 analogs.

Major efforts are still needed to improve the outcome of ITx that will likely remain part of the armamentarium required to prolong the survival of children with life-threatening complications of SBS. Nevertheless, the European experience has led to support of a more conservative approach more inclined to home PN, limiting referrals for ITx only to children with nutritional failure.

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Malnutrition

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Introduction

In 2018, close to 5.3 million children under 5 years of age died globally, with nearly 45% of these deaths attributable to undernutrition [1]. Undernutrition including stunting, wasting, and micronutrient deficiencies is a pervasive issue in the global under-five population, with an estimated 144 million children stunted and 47 million wasted (see Fig. 44.1). Around 14.3 million children fall into the severely wasted category. Conversely, the prevalence of childhood overweight and obesity is increasing worldwide (see Fig. 44.2), and a staggering 38.3 million children less than 5 years of age are overweight or obese [2]. Asia and Africa bear the biggest burden of all forms of under-five malnutrition with nearly 50% of all stunted children and the same proportion of all overweight children living in Asia. Lower-middle-income countries are struggling with this debilitating double burden of malnutrition in young children, adolescents, and adults. An estimated 64% of all stunted, 75% of wasted, and 37% of overweight children live in lower-middle-income countries [2]. If trends are not reversed, increasing rates of childhood underweight and overweight will have vast implications, not only for future health and healthcare expenditures but also for the overall development of nations.

The hidden burden of micronutrient deficiencies in young children heightens the risk of stunting, suboptimal cognitive development, reduced immunity, and frequent disease. Undernutrition, overweight, and micronutrient deficiency are now collectively termed as the triple burden of malnutrition. As per estimates, nearly 42% of all under-five children are anemic, with anemia often being used as a proxy for estimating iron deficiency [3]. Importantly, iodine insufficiency is the single biggest cause of brain damage and impaired cognitive development in children. Iodine deficiency in the pregnant mother can manifest as congenital hypothyroidism or cretinism in infants, which can affect 5–15% of the population in regions where iodine deficiency is endemic. Globally, deficiency of vitamin A affects nearly one-third of all children aged 6–59 months and is the leading cause of preventable childhood blindness. The highest rates of vitamin A deficiency in children are seen in sub-Saharan Africa and South Asia where nearly 48% and 44% of children 6–59 months, respectively, are affected [4]. Other notable micronutrient deficiencies are zinc and vitamin D. Zinc is critical for optimal growth and immunity. Given their higher nutrient requirements, children under 5 years of age are more likely to have a zinc deficiency. Regions identified as those

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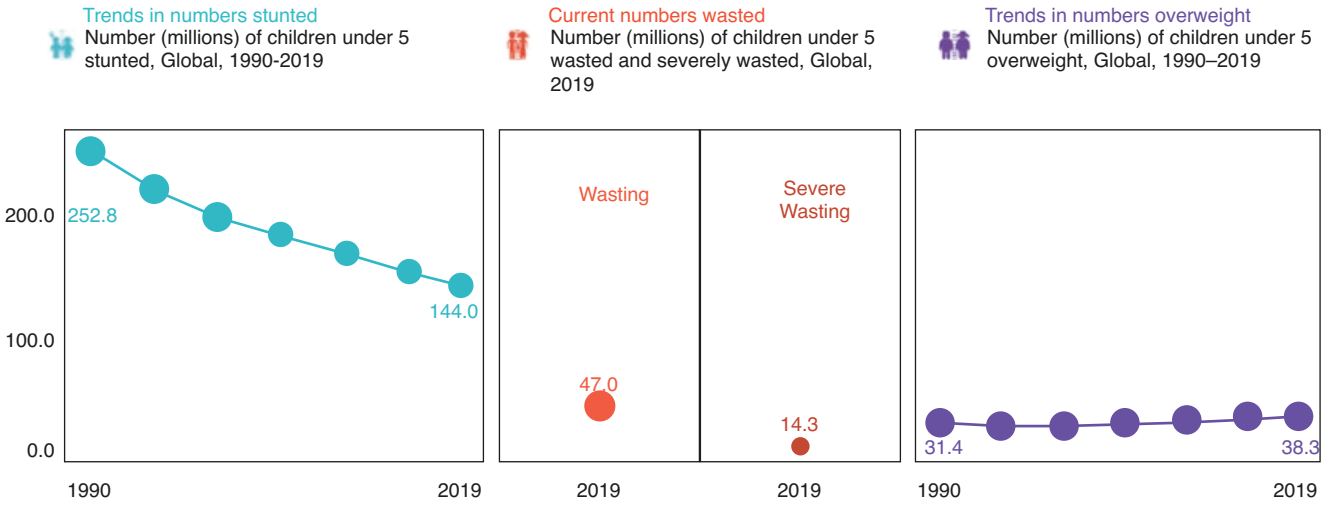

Fig. 44.1 Trends of stunting, wasting, and overweight from 1990 to 2019. (Source: UNICEF-WHO-World Bank: Joint Child Malnutrition Estimates – 2020 edition interactive dashboard. Available from https://public.tableau.com/profile/unicefdata#!/vizhome/JointMalnutritionEstimates2020Edition/Base (*Used with permission*))

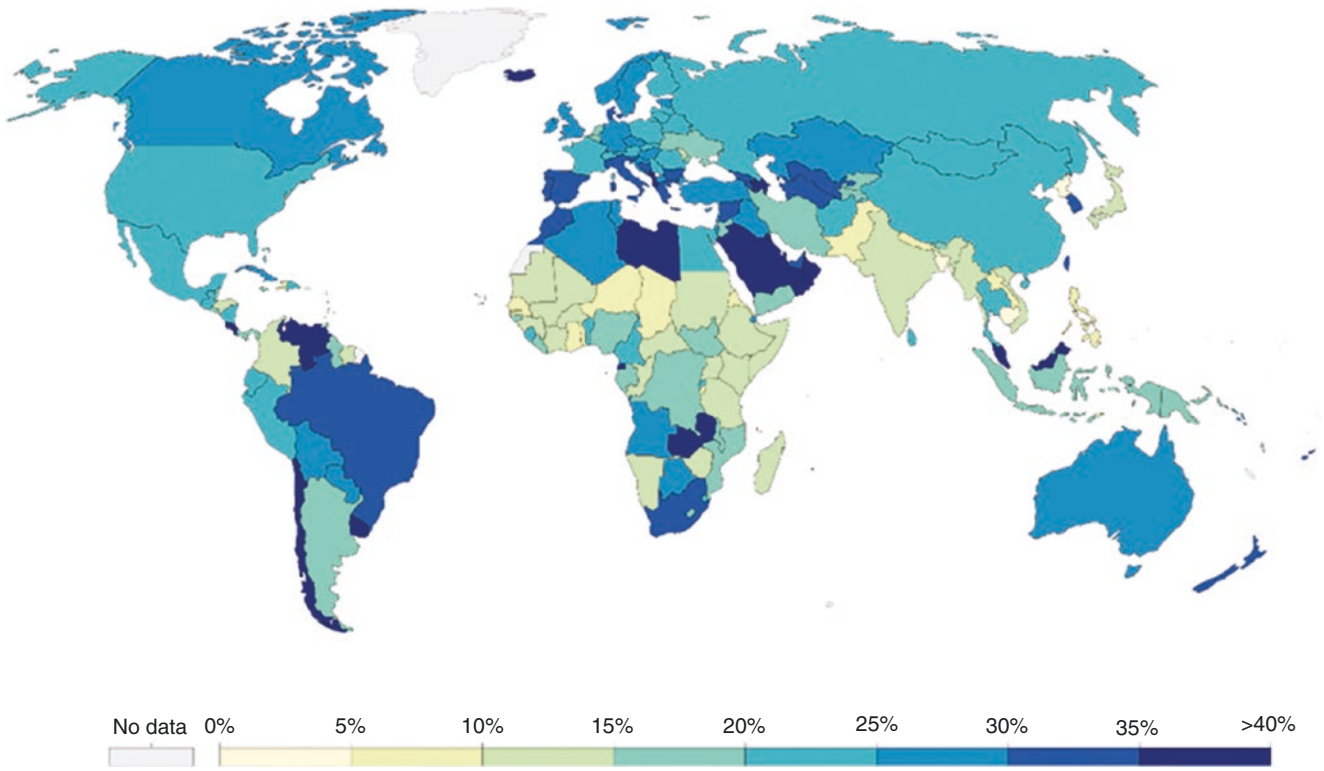

Fig. 44.2 World map of overweight or obese among children 2–4 years of age, 2016. (Source: Institute of Health Metrics and Evaluation (IHME). Available from https://ourworldindata.org/how-to-use-our-world-in-data (*Used with permission*))

with inadequate zinc intake are also often those with higher stunting prevalence.

Though considerable progress has been made in the last few decades, the world is still not on track to meet the 2025 global nutrition targets related to maternal, infant, and young child nutrition. The global exclusive breastfeeding rate of infants under 6 months stands at 42.2%, and substantial efforts will be needed to meet the target of a rate of 50% within the next 5 years. For under-five stunting, the global annual rate of reduction will need to be accelerated from the current 2.2% to at least 4.0% to achieve the 2025 target [5]. Alarmingly, many countries in Africa and Western Asia are

dealing with the double burden of malnutrition, with childhood stunting and overweight coexisting at the individual level, highlighting the importance of understanding and addressing the determinants of poor nutritional outcomes [5].

The impact of the COVID-19 pandemic will further impede and, even, reverse progress in many regions. With disrupted health systems, inconsistent access to food, and reduced coverage of interventions, childhood wasting is expected to increase during COVID and may account for 18–23% of additional under-five deaths [6].

School-Age and Adolescence

School-age (5–10 years old) is a phase of continued physical growth and rapid development. Nutritional deprivation and disease are common in this group and can manifest as wasting, underweight, stunting, and micronutrient deficiencies. Overweight and obesity are also a rising issue among this group. Adolescence (10–19 years) is a period of increased nutrient and energy needs for critical biological and psychosocial changes taking place. Importantly in this age group, adolescent girls, especially in low-resource settings, are at higher risk of undernutrition and iron deficiency anemia owing to menstrual losses, poor diets, and early pregnancies. Every year 12 million women aged 15–19 years give birth and nearly 800,000 more give birth before they are even 15 years old [7]. These young girls are at even greater risk of nutritional deficiencies, morbidity, and mortality.

Unfortunately, there are gaps in the nutritional data of these two groups, especially school-age children. Recognizing this evident gap, researchers are increasingly prioritizing the 5–19-year group. Though school-age children are at less risk of morbidity and mortality as compared to the first 5 years of life, poverty, increased nutritional needs, limited access to nutritious food, and frequent worm infestation lead to many nutritional issues such as stunting, iron deficiency anemia, and iodine deficiency in 5–10-year-olds. Iron deficiency is the primary cause of DALYs in school-age children and nearly 25% of all school-age children are anemic [8]. Further, evidence indicates that nearly 37% of all school-age children do not consume sufficient iodine which leads to impaired cognitive development.

Evidence indicates that in many countries nearly half of all adolescents are stunted, reflecting chronic undernutrition and growth retardation over the years. Data on the prevalence of underweight in adolescent females (13–17 years) shows it to be less than 5% in most countries with some exceptions such as Maldives, Vietnam, Sudan, and Cambodia where nearly 18%, 16%, 14%, and 13% of young girls (13–15 years) are underweight, respectively. Importantly, overweight and obesity are rising in this group with nearly 28% of adolescent females in the Eastern Mediterranean region being overweight, followed by Western Pacific (25%) and the Americas (25%) [9].

Iron deficiency remains the most common micronutrient deficiency in adolescents. Females, predictably, bear the greater burden of iron insufficiency and the consequent anemia, with South Asia and sub-Saharan Africa having the highest rates of iron deficiency in adolescents. Insufficiency of iodine is also a critical cause of DALYs lost in adolescent females and males especially in South Asia, sub-Saharan Africa, the Middle East, and North Africa. Other important micronutrient deficiencies in this age group include vitamin A, zinc, calcium, and vitamin D. There is growing evidence of vitamin D insufficiency in adolescents especially females, in many regions of the world including Europe, North America, and the Middle East. However, accurate data on the prevalence of micronutrient deficiencies in adolescents is limited.

Risk Factors

Risk factors for undernutrition and overweight range from broad national-scale determinants to individual specific and factors that affect various ages and periods of life. While there are factors distinct to specific age groups, many risk factors overlap.

Undernutrition is more than just a consequence of not eating enough food or illness. Numerous basic and underlying causes contribute to the development of undernutrition in under-five and school-age children and adolescents. National socioeconomic and political determinants have an impact and include political stability, economics, food security, poverty, and literacy among others. Natural disasters including famine, floods, and other emergencies have detrimental effects. Several social factors such as poor socioeconomic conditions, unstable contexts, limited access to care, poor living conditions, and household food insecurity exacerbate the issue of undernutrition. At a more intermediate level, suboptimal feeding practices including delayed initiation of breastfeeding, lack of exclusive breastfeeding, formula feed, delayed and inadequate complementary feeding, as well as poor WASH practices can all contribute to the poor nutritional status of under-five children.

Maternal factors such as maternal stunting, undernutrition, lack of education, poor nutrition during pregnancy, and consequent intrauterine growth restriction are associated with increased odds of stunting in the children [10]. Important to note is that lack of maternal education is associated with both increased childhood undernutrition and overweight, illustrating the significance of maternal education and awareness for optimal child nutrition.

Worrisome food insecurity is critical, but a factor that is potentially even more important in children is the inability to

absorb what they do take in because of repeated or persistent intestinal infections. Severe infectious diseases in early childhood, such as measles, diarrhea, pneumonia, meningitis, and malaria, can cause acute wasting and have long-term effects on linear growth. The most important of these infections is diarrhea, hence the need for understanding the impact and mechanisms of malnutrition and diarrhea, which form a vicious cycle of enteric infection worsening and being worsened by malnutrition. Several recognized processes by which enteric infections cause malnutrition range from well-recognized anorexia and increased catabolic or caloric demands to direct protein and nutrient loss or impaired absorptive function [11].

In older children, the same social factors also influence diets and nutrition status. Additionally, inadequate intake, nutrient-poor diets, unhealthy food choices, and repeated infections can lead to undernutrition and micronutrient deficiencies. Parasitic infections, most common in school-age children, also increase the risk of micronutrient deficiencies, particularly iron deficiency. Importantly, there are many common personal, lifestyle, environmental, and macrolevel factors and determinants that can lead to contrasting suboptimal nutritional outcomes, whether undernutrition or overweight and obesity, as illustrated in the causal factor framework (Fig. 44.3).

Adolescence is a period of rapid growth when maximum height and bone length is achieved. During the growth spurt, nutrient requirements may be double what they are during the rest of the adolescent period. These increased requirements put this group at a higher risk of undernutrition. Inadequate nutrition can affect physical growth and reproductive maturation. Nutrient-poor diets, such as junk food popular in this age group, and a lack of dietary diversity can lead to micronutrient deficiencies. Importantly, ongoing blood loss during menstruation puts adolescent girls at a higher risk of iron deficiency. Early marriages and pregnancies put them at even greater risk of undernutrition and micronutrient deficiencies.

The risk factors for being overweight and obese in childhood and adolescence are multifactorial. With suboptimal infant and young child feeding and increased consumption of processed and energy-dense food, the risk of overweight and obesity even in children under 5 years of age is increasing. Mixed feeding, use of formula milk, inclusion of processed foods in complementary diet, and a rising trend of giving sugar-laden drinks and snacks have increased the risk of obesity in infants and toddlers. Less physical activity and increased screen time escalate the probability of young children being overweight. Maternal obesity and gestational diabetes are also recognized as risk factors for infant obesity.

School-age and adolescent periods are formative years of life and habits formed during this time have lifelong implications. Children start becoming independent with many lifestyle influences outside the home environment, such as school, friends, and social media. Globally, in urban and rural communities, diets increasingly comprise high-fat, high-sugar, and high-sodium foods including fast food, ready-to-eat snacks, and soft drinks. Such foods are often

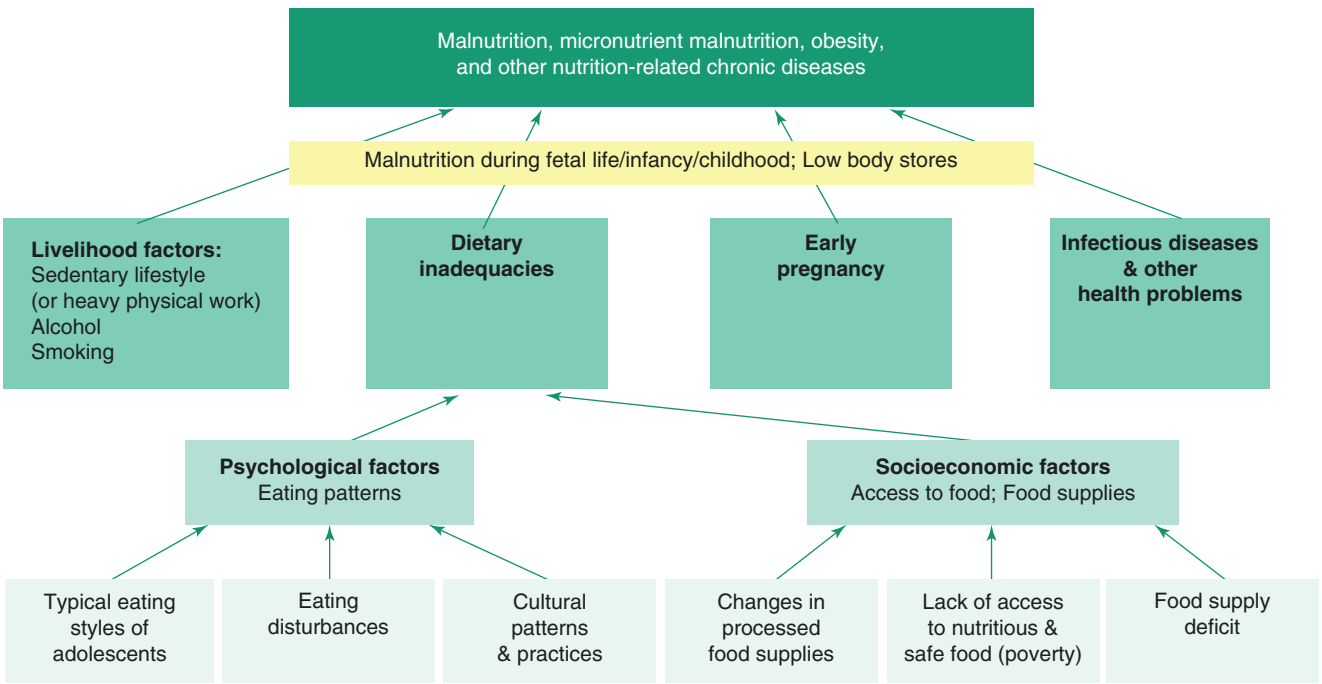

Fig. 44.3 Conceptual framework of nutritional problems and causal factors in adolescence. (Adapted from: WHO [12])

readily available and relatively cheap, making them tempting options, particularly for low-income families. Unhealthy diets, physical inactivity, and poor sleeping habits put older children at greater risk of overweight and obesity. A family history of overweight further increases the risk. The adolescent period is critical in terms of psychosocial development, and many adolescents face stress and depression which can lead to disordered eating such as binge eating, fad diets, skipping meals, and not eating at home – all behaviors that increase the risk of being overweight and unhealthy.

COVID-19 and Malnutrition: The impact of the COVID pandemic and the consequent response will undoubtedly affect the nutritional status of women and children in the long term. Modeling exercises estimate that with disrupted coverage of maternal and child health interventions and reduced food access wasting could increase by 10–50% leading to additional under-five deaths and maternal mortality. Additionally, experts predict a rise in micronutrient deficiencies, maternal undernutrition, and intrauterine growth retardation that would ultimately impact growth and increase childhood stunting [13].

Figure 44.4 summarizes the impact of COVID-19 on various drivers of maternal and child nutrition. Multiple factors, from basic drivers including diverted policies and redirected resources to poverty, limited food access, altered care-seeking behaviors, and unhealthy household environments, will most likely lead to poor nutritional intake and increased disease in populations.

As studies have shown, the effect of COVID-19 on household food security is not only an issue for low-income settings, with high-income settings also reporting nearly a 30% increase in household food insecurity, especially in vulnerable communities [14, 15]. Unemployment and suspension of school meal programs have led to an increased demand for food banks and food distribution programs.

Short- and Long-Term Consequences of Malnutrition on Growth and Development

Serious consequences of malnutrition can cause immediate disability but also long-term consequences that may manifest at later life stages. Additionally, cognitive, developmental, social, mental health, and economic consequences threaten the future economic productivity of these individuals impacted by malnutrition. As we have seen, the global burden of malnutrition is serious, with its impact potentially persisting throughout the life course for individuals, communities, and countries [16, 17]. In this section, the consequences are discussed for being underweight, overweight/obese, and having nutrient deficiencies.

Underweight

Being underweight is associated with impaired linear growth, impaired cognitive and motor function, weakened immunity, mortality, and various morbidities, sarcopenia, cardiac and renal dysfunction, and immunological defects. Childhood undernutrition may cause as many as 3.1 million deaths annually [18]. Adolescent undernutrition is also harmful to the health and wellbeing of individuals and societies and is also the beginning of one's reproductive life [19]. Being

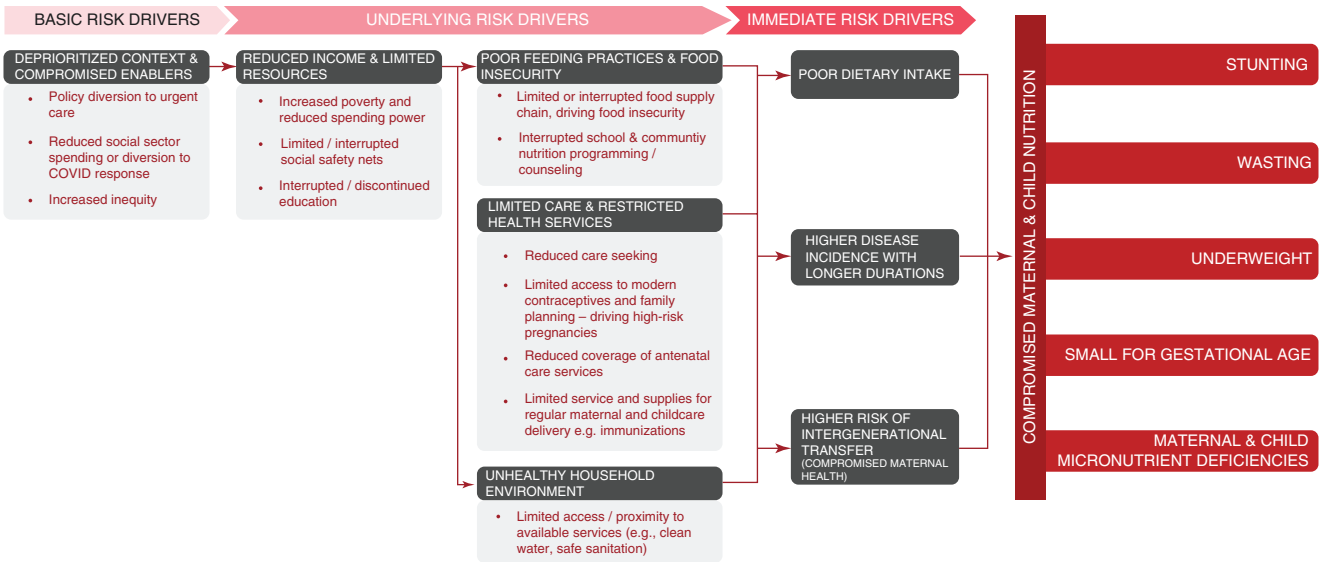

Fig. 44.4 Direct effects of COVID-19 on basic, underlying, and immediate drivers of acute and chronic malnutrition. (Source: Akseer et al. [13] (*Used with permission*))

underweight during adolescence may progress the burden of undernutrition into further generations [19].

Children Under 5 Years of Age

Underweight has negative effects on an individual possibly starting from before birth and lasting throughout the whole life course [20]. Birth weight is an important indicator for child health, and about 80% of newborns who die every year are low birth weight (LBW). This is because they were either born preterm, small for gestational age, or both [21]. LBW newborns who survive have a greater risk of both short- and long-term adverse health consequences than their normal-weight counterparts [21]. LBW has been associated with neonatal death, lower academic performance, long-term neurologic disability, impaired language development, reduced cognitive abilities, and increased risk of disease including cardiovascular disease (CVD) and diabetes [22]. Fetal conditions may also affect development and result in children being born SGA. Improper nourishment from the placenta during fetal development may result in intrauterine growth restriction (IUGR). IUGR places stress on the developing fetus and may result in metabolic syndrome later in life; however, not all SGA infants experienced IUGR. SGA infants, in general, may have more abdominal fat later in life and are at an increased risk of metabolic syndrome later in life, possibly due to catch-up growth and rapid refeeding in early life [23]. It has been suggested that early catch-up growth is needed for preterm infants to allow for proper neurodevelopmental outcomes, but this rapid weight gain is likely to affect the long-term health of LWB (and normal weight) infants [24].

Low birth weight, childhood stunting, and wasting all deplete components of metabolic capacity [25]. After birth, infant and child mortality is the most serious consequence of undernutrition. According to the WHO, 45% of all child deaths are linked to malnutrition [26]. A major driving force of infant mortality is the decreased immune function in underweight infants and children [27]. This immune dysfunction may result in illness from communicable diseases such as diarrhea and pneumonia and increase the risk of mortality [27]. Undernourished children may also have impaired liver, kidney, and thyroid function [28]. Furthermore, gastrointestinal changes in undernourished children include a stunted microbiome which may cause gut dysbiosis and increase the risk of intestinal infection [29]. Major cognitive, motor, and behavioral consequences are also important as brain development in early childhood may also be affected by protein-energy malnutrition. This includes a smaller brain size in malnourished children. The developmental abnormalities are likely to affect the infant socially, personally, and economically beyond infancy. In the long term, underweight children are also at risk of metabolic syndrome, central fat distribution, and obesity. This outcome is thought to be a major driver of the double burden of malnutrition. Although they are the most serious forms of undernutrition, even mild forms of undernutrition can have developmental effects as well as increase the risk of metabolic syndrome later in life [30].

School-Age Children (6–9 Years)

Undernutrition in school-age children also has serious long- and short-term consequences. Stunting is a marker of multiple pathologies associated with increased morbidity and mortality, loss of physical growth potential, reduced neurodevelopmental and cognitive function, and an elevated risk of chronic disease in adulthood [31]. The growth failure as well as being underweight may contribute to an increased lifetime risk of osteoporosis. As in infancy and early childhood, undernutrition puts an excess metabolic strain on the body which may increase the risk of metabolic syndrome and CVD later in life. The catch-up growth and rapid refeeding early in life may contribute to metabolic syndrome later in life [32, 33]. The immune system is also weakened with undernutrition, and these children may be more susceptible to communicable diseases and have more difficulties in recovering. Cognitive, motor, and behavioral delays persist into school-age if proper nutrition is not obtained. This undoubtedly has negative implications for school, learning, and academic achievement. This, in turn, has long-term economic consequences. Impaired intellectual development may be ameliorated when the malnutrition is resolved.

Adolescents (10–19 Years)

Significant gaps exist in the literature regarding adolescent malnutrition. Puberty which occurs during early adolescence increases nutritional requirements due to the period of rapid growth known as the pubertal growth spurt [34]. Adolescents may not reach their linear growth potential as this required increased nutrition to fuel a growth spurt [19]. Being underweight may also delay puberty [35]. The consequences for adolescent girls may persist into future generations as undernutrition and stunted growth may increase the risk for low birth weight in offspring [36]. Underweight adolescents also have an increased risk of osteoporosis [37]. Reduced cognitive capabilities and reduced work outputs may lower the academic achievement and work output of these adolescents as well. Collectively, these consequences may seriously reduce the economic productivity of individuals entering the workforce [38].

Overweight/Obesity

Excess body fat and overweight or obesity are important risk factors for many adult diseases. Being overweight in infancy, childhood, and adolescence is likely to persist for whole life [39]. Not only does this pose a significant risk to health, but

it also contributes to social, behavioral, and mental health problems. Collectively, these consequences contribute to a large economic burden in HIC and LMIC [40]. Obesity during childhood increases the risk for abnormalities in cardiovascular function, glucose homeostasis, pulmonary function, and cognition during childhood, as well as risk for obesity (and therefore mortality) in adulthood [18].

Children Under 5 Years of Age

The most important risk factors for being overweight or obese in children under 5 years of age are those that occur early in life. High birth weight has been associated with an increased metabolic load which may increase the risk of metabolic syndrome later in life [41] and a higher risk of hypoglycemia after birth and birth defects [42]. High birth weight has also been suggested as a risk factor for developing leukemia, obesity in childhood and adulthood, and later CVD [43]. Although high birth weight may have negative consequences, low birth weight remains a more important risk factor for many morbidities and all-cause mortality [43].

School-Age Children (6–9 Years)

Arguably, the most important consequence of childhood obesity is the increased disease risk in adulthood. Overweight children often remain overweight adults [44]. Aside from long-term, chronic disease risk, there are also immediate consequences in childhood. These include early type 2 diabetes or prediabetes, fatty liver disease, sleep apnea, type 2 diabetes, asthma, hepatic steatosis (fatty liver disease), CVD, high cholesterol, cholelithiasis (gallstones), glucose intolerance and insulin resistance, skin conditions, menstrual abnormalities, gastrointestinal reflux, impaired balance, and orthopedic problems [45]. The social and psychological health effects add to the burden as obese children are at risk of depression, anxiety, bullying, and low self-esteem [46]. Because low birth weight and catch-up growth may contribute to childhood overweight, the double burden of malnutrition is rapidly increasing across the globe.

Adolescents (10–19 Years)

Adolescent obesity, as with childhood obesity, leads to an increased risk of morbidity and mortality due to cancer, CVD, and metabolic diseases in adulthood. In this period that encompasses puberty, overweight can influence the progression of pubertal milestones [35]. Multiple studies have shown that the secular trend of puberty timing in girls is related to the increasing trend of childhood obesity [47, 48]. This may be related to catch-up growth in infancy and childhood [49]. Obesity in peripubertal girls may also be associated with hyperandrogenemia and a high risk of adolescent polycystic ovary syndrome, which is the leading cause of infertility in women, and also increases the risk of metabolic syndrome, diabetes mellitus type 2, CVD, and endometrial cancer. In boys, obesity may delay puberty but there is less evidence than in girls [50]. There are also important mental health and behavioral consequences in this period of change including anxiety, depression, lack of self-esteem, lower quality of life, as well as social problems that include bullying and a lower lifetime earning potential [19].

Nutrient Deficiencies

Micronutrient deficiencies are a major problem across the globe and can pose a threat to the long-term health of infants, children, and adolescents. Micronutrient deficiencies may be associated with stunted growth, cognitive delays, weakened immunity, and other morbidities [51]. For pregnant women, the lack of essential vitamins and minerals can increase the risk of low birth weight, birth defects, stillbirth, and even death. Clinical manifestation of micronutrient deficiencies can be devastating.

Children Under 5 Years of Age

Micronutrient deficiencies can start in fetal life if the mother is malnourished. Zinc, iron, and iodine deficiencies are very prevalent in pregnant women [52]. These deficiencies can result in abnormal fetal development [52, 53] and increase the infant's risk of chronic disease due to altered organ development, size, and function. Vitamin A deficiency in pregnancy may cause preterm birth which is a risk for child mortality. Iron, iodine, and vitamin A deficiencies in infancy and childhood have serious and lifelong consequences and remain common in many LMICs. In early development, vitamin A and iodine are crucial for brain development. Iodine deficiency remains the most important cause of avoidable brain damage [54]. Iodine deficiency can cause abnormal myelination of neurons, hearing impairment, and hypothyroidism. This deficiency is of crucial importance as there are long-term economic impacts when a child fails to meet intellectual capacity. Iron deficiency causes impaired reactivity and coordination in infants: psychomotor and mental development [55] may persist with iron therapy which is why preventing deficiency is needed. A lack of sufficient iron intake may significantly delay the development of the central nervous system as a result of alterations in morphology, neurochemistry, and bioenergetics [56]. Vitamin A deficiency increases the risk of respiratory and diarrheal infections, growth failure, night blindness, and permanent blindness. Vitamin A deficiency can contribute to stunting and infant mortality [57, 58]. Additionally, retinoic acid is critical for brain development, and a deficiency is associated with learning and memory problems and decreased plasticity of the hippocampus. Zinc deficiency impairs immune, nervous, and endocrine functions, increases the susceptibility to infection, and restricts physical growth [59]. Vitamin D deficiency pre-

vents bone mineralization and causes rickets which may cause permanent changes to bone structure. In this period of rapid growth, adequate vitamin D is needed. Vitamin D is also needed for optimal nerve, muscle, and immune function [60]. Micronutrient deficiencies threaten health in the long and short term and hurt the economy of countries with widespread micronutrient deficiency [61].

School-Age Children (6–9 Years)

Children in this age group share the consequences for micronutrient deficiencies observed in early childhood. Iron is important for cognitive development. It is also needed for proper musculoskeletal growth. The most common cause of anemia is iron deficiency, and anemia can decrease school performance, productivity in adult life, quality of life, and the general income of affected individuals [62]. Vitamin A is still needed in school-age children for immune function and preventing night blindness [63]. Furthermore, iodine deficiency may affect cognition by disturbing normal brain development, and it may affect cognition by altering brain function possibly due to altered thyroid function [64]. The maturation of brain regions responsible for higher cognitive function continues to mature in childhood and adolescence.

Adolescents (10–19 Years)

Delayed growth, goiter, increased risk of infection, blindness, anemia, and inadequate bone mineralization, along with other psychological, social, and economic consequences, remain a concern in adolescents with micronutrient deficiency. Iron deficiency, especially common in adolescent girls, has adverse effects on productivity and cognition in the general population and is the leading cause of anemia during pregnancy, contributing to 20% of all maternal and perinatal mortality and low birth weight [65]. Zinc deficiency in adolescence may alter sex hormone metabolism and impair growth and immunity [38]. Vitamin D is needed in this period of growth for bone growth, strength, and development. Vitamin D deficiency may reduce bone density and increase the lifetime risk of osteoporosis, fractures, and decreased quality of life during later life stages [66]. Iodine and vitamin A continue to be needed for brain development, immunity, growth, and eye health [67].

Interventions

The causes of malnutrition are complex and multifaceted; therefore, improving childhood malnutrition requires double-duty actions to address the double burden of malnutrition which occurs when undernutrition coexists with overweight, obesity, and diet-related non-communicable diseases [68]. Such interventions have also been categorized as nutrition-sensitive or nutrition-specific interventions [69]. Some examples of nutrition-specific interventions for malnutrition include food fortification and supplementation, while nutrition-sensitive interventions include education, clean water, sanitation, agriculture programs, regulatory interventions, and deworming [70]. Generally, interventions appear most successful where there is a strong political commitment along with collaboration between government, nongovernment, national, and international organizations [71].

Age Group 0–5 Years

Neonatal Interventions

Widespread interventions to reduce the risk of LBW, SGA, and preterm birth involve improving women's preconception/conception nutritional status. Anti-malarial drugs and maternal nutrition education have also been shown to benefit neonatal outcomes [72, 73]. The benefits of preconception/conception supplementation are nuanced, but a few systematic reviews have demonstrated that oral supplementation with vitamin A, low-dose calcium, zinc, or MMN (multiple micronutrients) can have a considerable effect in decreasing LBW, SGA, and preterm birth (PTB) [73–75]. Vitamin A with other micronutrients (iron plus folate) reduced LBW by 33% versus micronutrient supplements without vitamin A [74]. However, vitamin A alone did not reduce LBW compared with placebo or no treatment. Oral low-dose calcium supplementation (less than 1 g/day) significantly reduced LBW by 80% but did not affect the risk of SGA [76]. Oral zinc supplementation in adolescent pregnancy reduced the likelihood of LBW by 61% as evidenced by a systematic review of five RCTs [77]. A significant reduction of LBW in the range of 11–14% and a significant reduction of SGA in the range of 10–17% were observed following multiple micronutrient (MMN) supplementation [75]. However, when comparing MMN containing iron and folic acid versus placebo, the effect was not statistically significant. In the aforementioned trials, the effect on PTB was unaffected or not statistically significant. A systematic review by Muanda et al. (2015) suggests that women receiving anti-malarial drugs have also been shown to have a reduced risk of LBW 27% compared with women not receiving these drugs during pregnancy [78]. The intervention did not affect the risk of PTB and other outcomes were not reported. Finally, nutrition education LBW was reduced by 96% and PTB by 54% for women receiving nutritional education to increase energy and protein intake compared with no nutritional education in pregnancy as evidenced by a systematic review [72]. No effect on SGA was seen for this intervention. Other interventions did not have a meaningful effect on these neonatal outcomes.

Breastfeeding Interventions

In children 0–5 years of age, breastfeeding and effective complementary feeding are of paramount importance to ensure optimal nutrition and thus proper growth and development. All-cause mortality, as well as infection-related mortality, was lower in exclusively breastfed infants (0–5 months of age) as opposed to predominantly, partially, and non-breastfed infants [79]. Additionally, children 6–11 and 12–23 months of age who were not breastfed had 1.8- and 2.0-fold higher risk of mortality, respectively, when compared to those who were breastfed. The risk of infection-related mortality was double in non-breastfed children when compared to breastfed children aged 6–23 months. Fortified breast milk interventions improved short-term growth in weight, length, and head circumference in preterm infants compared to unfortified or micronutrient fortified breast milk, while not increasing the risk of necrotizing enterocolitis or feed intolerance [80]. In preterm and LBW children unable to obtain breast milk from their mother, a systematic review by Quigley and colleagues demonstrated that feeding with formula compared with donor breast milk resulted in higher rates of weight gain, linear growth, and head growth but had no effect on all-cause mortality or on long-term growth or neurodevelopment [81].

Breastfeeding rates are far from optimal despite its clear benefits [82–85]. Breastfeeding interventions can be effective and have higher improvements in breastfeeding rates when delivered in a combination of settings [85]. Greatest improvements in early initiation of breastfeeding were achieved when counseling or education was provided in home and community, exclusive breastfeeding in health systems and community, and continued breastfeeding rates in health systems and home settings with the most effective interventions being support at the health system such as level baby-friendly hospital environments [85]. Interestingly, a 2015 systematic review and meta-analysis found no significant effect of breastfeeding promotion interventions on child growth on length or height z-scores [86].

Complementary Feeding Interventions

The WHO complementary feeding guidelines recommend that infants at about 6 months should be introduced to nutritional foods in addition to breastfeeding [87]. Improving complementary diets of children aged 6–23 months is a recommended approach for reducing stunting in children under 5 years old, but the potential of these interventions to prevent wasting is inconclusive [88]. Complementary foods containing iron (naturally or fortified) help prevent iron deficiency and maintain iron status in the first year among infants at risk of insufficient iron stores (but not infants with already low iron stores) [89]. A recent Cochrane review [90] was unable to confirm any benefit of a meat-based intervention compared to a dairy-based intervention or fortified cereals on linear growth and weight gain.

Stunting, Wasting, and Underweight Interventions

Interventions most commonly implemented for childhood stunting, wasting, and underweight include supplementation, nutrition education and counseling, growth monitoring and promotion, immunization, water, sanitation and hygiene, and social safety nets [71]. As evidenced by a systematic review of children 6–24 months of age in LMICs [91], iron plus folic acid supplementation and multiple micronutrients improve height-for-age z-score (HAZ) but only MMN reduces the risk of stunting. This review also found that flour in the caloric range of 270–340 kcal and fortified lipid-based nutrient supplements containing 220–285 kcal decreased the risk of stunting compared to standard of care, but these interventions and other food supplements did not show improvements for HAZ [91].

Water, Sanitation, and Hygiene (WASH) Interventions

Hygienic conditions prevent diarrhea and parasitic infections which could cause reduced absorption and nutrient losses, reduced appetite, and diversion of energy and nutrients from growth to the immune system [92, 93]. Repeated intestinal infection may also cause environmental enteropathy which increases the small intestine's permeability and reduces nutrient absorption [94]. WASH interventions alone improved HAZ when delivered over 18–60 months and for children under 2 years of age as evidenced by a systematic review [95]. This review also showed that interventions combining WASH with nutrition had a strong effect on stunting and underweight (weight for age z-score) and a modest effect on wasting. Thus, a synergistic effect of WASH and nutritional interventions is of interest. Another systematic review [96] also linked WASH interventions to increased mean HAZ. Additionally, a systematic review that studied results in LMICs [97] showed diarrhea risk reductions between 27% and 53% in children 0–5 years old, depending on the WASH intervention type.

Micronutrient Deficiency Interventions

Interventions for stunting, wasting, and underweight overlap with many micronutrient deficiency interventions. Micronutrient supplementation is the most popular intervention for both single micronutrient and multiple micronutrient deficiencies. According to a 2019 review of systematic reviews, the most effective strategies with the clearest evidence for addressing micronutrient deficiencies in children 0–5 include optimal cord clamping, anthelmintic treatment, anti-malarial treatment, and supplementation of single or

multi-micronutrients [98]. Delayed cord clamping is an effective intervention for reducing anemia in early life. In helminth endemic areas, iron status can be improved by anthelmintic treatment, and anti-malarial treatment can improve ferritin. In deficient populations, single iron, vitamin A, and MMN supplementation can improve iron, vitamin A, and multiple micronutrient status, respectively. While the impact of home fortification on multiple micronutrient status remains questionable, commercial iron fortification may improve iron status [98].

Food Fortification Interventions

Food fortification may be one of the most cost-effective and safe strategies to reach populations at large as deemed by the Copenhagen consensus [99]. Home fortification is preferred over commercial fortification from a cost perspective [100]. However, evidence from a review of systematic reviews indicates that multi-micronutrient home and commercial fortification did not significantly increase HB, serum ferritin, zinc, or vitamin A consistently [98]. Another systematic review [101] does not show a clear benefit of point-of-use multiple micronutrient fortification for a child or maternal anemia and hemoglobin.

Iron Supplementation and Fortification Interventions

Iron supplementation and fortification have been widely proven at the systematic review level to improve ferritin and hemoglobin concentration [98]. Delayed cord clamping has been shown to improve the hematological and iron status of both preterm and term infants after the neonatal period, as evidenced by a systematic review [102]. Systematic review evidence shows that malaria treatment increases serum ferritin but does not decrease the risk of anemia after 12 weeks [103]. A systematic review by Neuberger and colleagues (2016) found that iron status was improved the most in malaria-endemic areas when iron supplementation was combined with anti-malarial treatment [104].

Vitamin A Supplementation and Fortification Interventions

Vitamin A supplementation interventions not only reduce the risks associated with vitamin A deficiency but have also been associated with a reduction in the risk of all-cause mortality [105].

Zinc Supplementation and Fortification Interventions

Zinc supplementation and fortification have been shown to increase serum zinc concentration in several reviews [106–108]. Petry and colleagues (2016) found that low-dose daily iron and zinc use during 6–23 months of age has a positive effect on child iron and zinc status [109].

Intervention to Decrease Bodyweight

In children under 5 years of age, feeding is influenced and, in most cases, controlled by parents, school, and other authorities making community and family interventions important for preventing childhood overweight and obesity. Breastfeeding, along with its aforementioned benefits, also may lower rates of obesity later in life. Other chronic diseases that are reduced by breastfeeding include diabetes (both type 1 and type 2), obesity, hypertension, cardiovascular disease, hyperlipidemia, and some types of cancer [110]. There is no conclusive evidence, at a systematic review level, of when the best time to introduce complementary foods to reduce the risk of overweight [111]. A systematic review [112] found home-based interventions with family involvement, preschool/early childhood settings, multicomponent interventions across multiple settings, and healthcare settings as possible options. A 2019 Cochrane review by Bown and colleagues suggests that interventions that include diet combined with physical activity interventions can reduce the risk of obesity in children aged 0–5 years, while physical activity interventions only do not have a significant effect [80]. A community approach to these interventions was shown to be the most useful for the overweight. This review also suggests a promising role for increasing the accessibility of education on diet and physical activity for disadvantaged families to prevent childhood obesity while making the interventions culturally acceptable. Another systematic review [113] found that school-based interventions are effective in reducing excessive weight gain of children. This review found significant results for the effectiveness of single-component intervention (physical activity), but emphasizing the enjoyment of physical activity sessions was critical for the interventions. Preschool interventions should be an avenue of intervention as food choices are shaped at this early age.

School-Age Children (6–10 Years)

Antihelminthic Interventions

Malnutrition research is abundant regarding children under 5 years; however, school-age children are an important group of interest to reduce the burden of malnutrition. An intervention for school-age children is routine treatment with antihelminthic drugs. One systematic review [114] shows evidence of an association between helminth infections and micronutrients (mostly iron and vitamin A) in school-age children. However, evidence from two systematic reviews, one by Taylor-Robinson and colleagues [115] and one by Welch and colleagues [116], does not justify treating all schoolchildren at regular intervals with deworming drugs in areas where helminth infection is common. Although deworming has not demonstrated meaningful improvements in height, hemoglo-

bin, cognition, school performance, or mortality, it has demonstrated modest increases in weight; nevertheless, the WHO recommends widespread deworming [117].

Supplementation Interventions

Iron supplementation safely improves hematologic and non-hematologic outcomes among primary-school-age children in low- or middle-income settings and is well-tolerated [118]. A systematic review of studies among children 0–10 years of age [119] showed that zinc supplementation may modify fat-free mass among children with pre-existing growth failure.

Fortification Interventions

Large-scale food fortification (LSFF) of staple foods to prevent micronutrient deficiencies has been shown to have significant improvements in the micronutrient status of children despite programmatic and implementation limitations [120]. Other hematologic markers also improved following food fortification among children with vitamin A, iron, and MMN, suggesting some conjoint benefits. MMN fortification, when compared to placebo/no intervention, has demonstrated reductions in anemia, iron deficiency anemia, and micronutrient deficiencies (iron, vitamin A, vitamin B2, and vitamin B6), but MMN fortification was not associated with any significant benefits on HAZ, WAZ, and WHZ [121]. The fortification of foods with vitamin D has also been associated with improvements in the vitamin D status of children aged 2–11 years [122]. Additionally, vitamin D food fortification has also been associated with improved blood concentrations, deficiency prevention, and improved IQ levels [123].

Intervention to Decrease Bodyweight

A recent Cochrane review highlighted the importance of this age group for overweight and obesity interventions [124]. This review found that interventions with physical activity alone or in combination with diet can decrease the risk of obesity for this age group (no evidence was found for the effectiveness of diet only). Multicomponent behavior-changing interventions that incorporate diet, physical activity, and behavior change may be beneficial in achieving small, short-term reductions in BMI, BMI z-score, and weight in children aged 6–11 years as evidenced by a systematic review [125]. However, sustained reduction in these parameters is necessary to reduce the burden of overweight in children. Interventions for overweight and obesity in this age group primarily target diet and physical activity and are typically school-based, community-based, or home-based interventions. School-based interventions with combined diet and physical activity components and a home element had the greatest effectiveness in a systematic review of children of preschool and school-age children [17]. Multicomponent lifestyle interventions have been consistently shown to have a beneficial effect on weight loss and obesity prevention. Evidence on the use of multicomponent interventions in the treatment of childhood overweight and obesity was shown in a 2017 systematic review [126].

Adolescence (10–19 Years)

Underweight and Micronutrient Deficiency

Nutrition interventions specifically for adolescents seem to be less important for boys, whereas iron and MMN supplementation may have meaningful benefits for adolescent girls. A systematic review by Salam and colleagues [127] identified the most pertinent interventions for adolescent nutrition. This study primarily found evidence suggesting an overall significant reduction in anemia with iron/iron-folic acid supplementation (primarily in females) alone or in combination with other micronutrient supplementation. Furthermore, nutrition strategies, mainly consisting of calcium and zinc supplementation, among "pregnant adolescents" showed statistically significant improved birth weight, decreased low, and preterm birth. Other interventions that studied the effect of various micronutrient supplementation/fortification on body mass index (BMI) and MMN fortification did not provide certain evidence. Secondarily, iron supplementation irrespective of folic acid may improve hemoglobin concentrations, and calcium/vitamin D supplementation may improve serum vitamin D levels, and body bone mineral density may be marginally improved with calcium-only supplementation and calcium and vitamin D supplementation. These results mirrored a systematic review by Lassi and colleagues [67] that suggested that iron alone, iron plus folic acid, zinc, and MMN supplementation in adolescents can significantly improve serum hemoglobin concentration [67]. It also suggested that zinc supplementation in pregnant adolescents showed improvements in preterm birth and low birth weight. Regarding underweight adolescents, there was limited evidence on food/protein energy supplementation in adolescents. Overall, studies have found limited evidence supporting micronutrient supplementation/fortification among adolescents on health and nutritional status [67, 127].

Intervention to Decrease Bodyweight

Adolescent obesity is multifaceted with a lack of guidelines for proven weight loss success interventions. Multidisciplinary interventions which include family support and guided behavior modification appear to be one type of effective method to combat adolescent overweight and obesity [128]. Additionally, a recent Cochrane review suggested the beneficial impacts from programs that combine the promotion of healthy dietary habits with physical activity on preventing obesity in children and adolescents, especially school-based

programs [124]. The absence of clearly proven strategies to prevent or treat obesity demonstrates the complexity of the etiology of obesity [129].

Conclusions

Commitments made by countries to the Millennium Development Goals (MDGs) have significantly helped decrease maternal and infant mortality and childhood stunting. The Sustainable Development Goals (SDGs) aimed to build on those successes while expanding goals to adolescents who were largely ignored in the MDGs [130].

Supporting adolescent policies in addition to childhood nutrition policies and strategies represent important targets to improve malnutrition among children and adolescents. For almost two decades, reports have highlighted the need for expanded global health programs and policies targeting adolescents [131, 132]. In response to this *call for action*, the SDGs included 90 youth-related indicators [130]. The latest Sustainable Development Report, however, only identifies two SDG goals that are on track to achieve Goal 1 (No Poverty) and Goal 13 (Climate Action) [133]. Adolescent nutrition guidelines in many LMIC are limited in scope as they focus on school feeding, obesity, and supplementation for some micronutrients [134].

Continued gains in decreasing childhood and adolescent malnutrition will be negatively impacted by COVID 19. Concerted efforts will require greater national nutrition programs and enhanced intersectoral approaches among government and nongovernment agencies to overcome these new challenges.

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Enteral Nutrition

45

Mora Puertolas and Timothy A. Sentongo

Introduction

Enteral nutrition (EN) or enteral tube feeding (ETF) as currently practiced is a technique for nutritional support which delivers a homogeneous, liquid nutrition admixture into the digestive tract by tube, into the stomach, duodenum, or the proximal jejunum. The inception of enteral feeding dates back to 3500 years ago in ancient Egypt, when practitioners used rectal enemas to administer wine, milk, broth, and other nutrients to the ill [1, 2]. The first documented delivery of EN through a tube inserted into the esophagus was by Capivavacceus of Venice in 1598, who constructed a device from animal bladder. Recognition of the importance of nutrition therapy during injury and recovery from disease rapidly grew in the 1930s and 1940s and led to development of specialized commercial enteral feeding products in the 1950s and modern EN formulas in the 1970s [1, 2]. In 1980, Guaderer and colleagues at Rainbow Babies and Children's Hospital in Cleveland, Ohio, USA, were the first to describe the technique of inserting feeding gastrostomy tubes (GT) without requirement for laparotomy, that is, percutaneous endoscopic gastrostomy (PEG) tube [3]. EN may be administered via oral, nasal, gastrostomy, or jejunal routes (Fig. 45.1).

EN is used to preserve nutritional status, support normal growth, and treat malnutrition when oral feeding is inadequate or not possible. EN is more physiologic, usually safer, easier to administer, and less costly compared to parenteral nutrition (PN). Therefore, EN should be preferred to PN in infants, children, and adolescents with malnutrition and/or nutritional risk, when the intestinal tract is usable to provide sufficient nutrients for achieving optimal growth or catch-up growth [4]. EN may be administered rapidly as a bolus into the stomach, or more slowly over several hours as a continuous infusion into the stomach, duodenum, or jejunum. The underlying disease and patient tolerance are what determines whether to use bolus or continuous feeds. The physiological basis of continuous EN makes it of great interest in pediatric patients with feeding intolerance and other gastrointestinal (GI) disorders [4]. EN may be used as the sole source of nutrition or to just supplement a patient's oral and/or PN intake. Also, depending on the indication, EN may be used daily or just on a periodic basis. While EN is normally initiated in the hospital, continuing it at home has become a common option. Home EN may be used long term or just as a temporary bridge until children achieve oral food intakes that support adequate growth and nutritional status. Even though home EN is an effective method of meeting a child's growth and nutritional requirements, health practitioners should not disregard the mixed acceptance by families and sometimes negative impact on quality of life [5–7].

Physiological Basis of Continuous Enteral Feeding

Gastrointestinal Motility

The rate of gastric emptying, and secretion of pancreatic biliary fluids, is regulated by the infusion rate, calorie density, and osmolality of the enteral feeds [8]. In the case of gastric administration of continuous feeds, a rate of continuous gastric emptying related to the infusion rate can be achieved if the infusion rate, calorie density, and osmolality of the mixture are not excessive. Steady-state gastric emptying of 1 kcal/mL formula can be maintained at infusion rates of ≤3 mL/min. When the infusion rate is excessive and higher than the gastric emptying rate, the risk of vomiting increases. As caloric load and/or osmolality of the formula increases, the gastric emptying rate is reduced to maintain a constant calorie load delivered into the duodenum. Thus, EN

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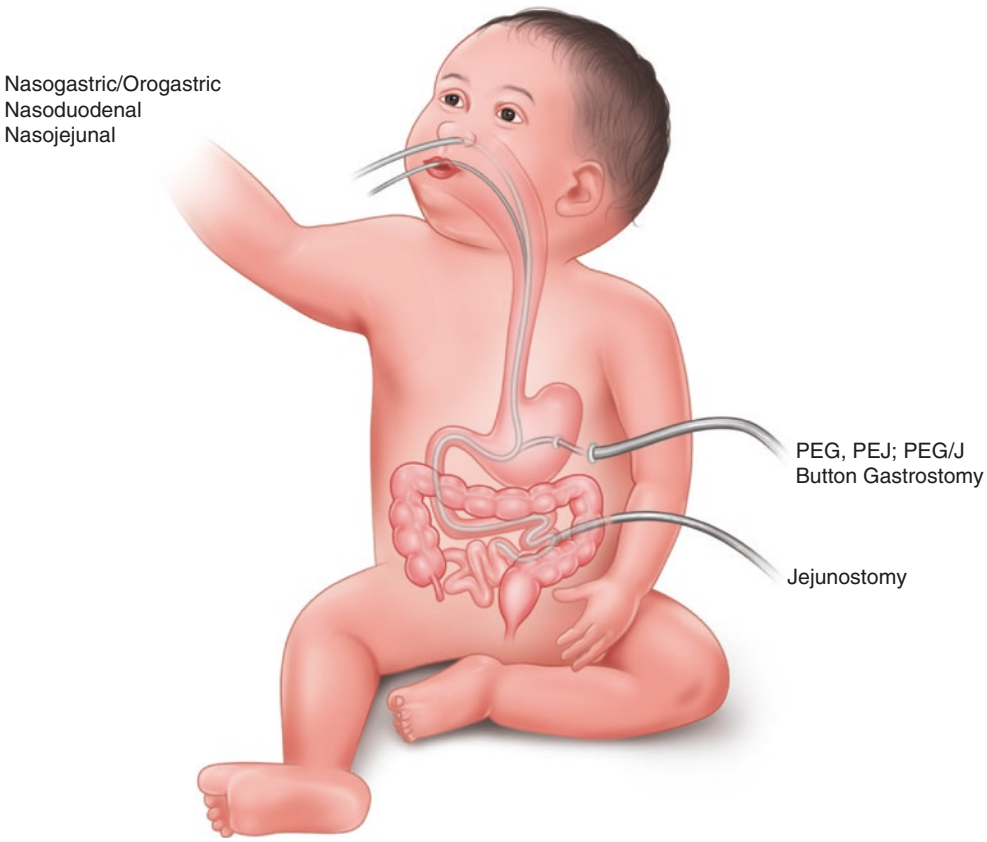

Fig. 45.1 Possible routes for EN

PEG: Percutaneous endoscopic gastrostomy tube
PEJ: Percutaneous endoscopic jejunostomy tube
PEG/J: Percutaneous endoscopic gastrostomy jejunal tube

consisting of very calorie-dense formulas should initially be administered very cautiously. The individual nutrient composition of the formula has a lesser effect on gastric emptying function than the caloric density, except for the type of triglyceride: long-chain triglycerides (LCTs) cause greater delays in gastric emptying than medium-chain triglycerides (MCTs).

Gastric emptying is dependent on duodenal function. The effects of continuous enteral nutrition (CEN) on intestinal motility can be analyzed by manometry. Motor migrating complexes are observed in adults during CEN, as during fasting state [9]. In small preterm infants, duodenal motor activity is higher following slower infusion of gastric feeds than with rapid boluses, and this is associated with lower postprandial gastric contents [10]. During administration of jejunal feeds, energy loads at rates within the physiologic range of gastric emptying (≤4 kcal/min) initiate normal-motor small-bowel motor responses; however, increasing the osmolality (>600 mosmol) has a significant inhibitory effect on small-bowel contractile and propagative activity [11] and thus greater likelihood for intolerance. Very few data are available about the changes of colonic motility induced by CEN; however, the continuous gastric infusion of nutritive formula modifies the gastrocolic reflex. Gallbladder motility is maintained during CEN as assessed by increased serum cholecystokinin (CCK) and ultrasonography [11, 12]. Emulsified LCTs delivered to the duodenum have a potent stimulating effect on CCK release and gallbladder contraction [13]. Conversely, whereas dietary MCTs are more efficiently absorbed and rapidly metabolized compared to LCTs [14], they are very weak stimulants for CCK release, gallbladder contractility, and hence luminal postprandial bile acid concentrations. Biliary complications such as sludge or cholelithiasis are rare during long-term CEN.

Digestive Secretion and Hormonal Response

Gastric secretion depends mostly on protein intake and, in the case of elemental diet, on amino-acid composition. Gastric secretory response is reduced by lipids and not influenced by carbohydrates. It has not been demonstrated whether or not the type of diet (i.e., elemental, semi-elemental, or polymeric) modifies gastric acid secretion [15]. Secretion of CCK and pancreatic polypeptide (PP) is maintained during CEN [12]. All forms of oral and enteral feeding

stimulate pancreatic synthesis and secretion of fluids and enzymes through CCK, secretin, and PP. Pancreatic secretions can be reduced by 50% if a low-fat elemental formula is used for duodenal feeding. Stimulation of pancreatic trypsin synthesis or secretion can be inhibited by delivering EN into the mid-distal jejunum [16]. The mechanism involves increased secretion of the "ileal-brake" hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) [17, 18] which inhibit production of pancreatic secretions and motility in the proximal GI tract. Gastrin secretion is also maintained during CEN, but its response to protein load is decreased. Gastric or duodenal CEN stimulates insulin secretion depending on the type of infused nutrients. The glycemic and insulinemic response induced by EN parallels that of oral feeds and is significantly lower than PN [16]; therefore, less risk for steatosis.

Effects of CEN on Mucosal Trophicity

The effects of elemental formulas (totally absorbed in the upper GI tract and providing minimal residue to the distal bowel) on small-bowel mucosal trophism remain controversial. In studies comparing intestinal trophicity and function in animals fed elemental diets versus regular chow, there was similar digestive function but significantly decreased mucosal mass in the distal bowel of animal-fed elemental diets. These changes could be the consequence of the almost complete absorption of nutrients within the proximal part of the small bowel, leading to lack of stimulation of the distal segment [19, 20]. This suggests an ability of EN to achieve bowel rest in the distal part of the bowel, providing efficient treatment for ileocolic inflammatory diseases.

Effects of CEN on Energy Expenditure and Feeding Tolerance

Thermogenic effect of feeding is related to the increase of energy expenditure for synthesis and secretion of digestive enzymes following ingestion of food. The increase in energy expenditure induced by CEN in normal subjects is lower than when the same nutrients are administered by bolus feed [21]. Finally, the slow and continuous administration of nutrients into the GI tract through CEN allows the achievement of optimal utilization despite intestinal illness. In fact, by changing the conditions of flow and of contact between the nutritive formula and the digestive tract, CEN may increase the capacity for intraluminal digestion and intestinal absorption. This feeding technique does not appear to provide benefit in patients without intestinal disease [22, 23]; however, it seems logical and efficient when the absorptive surface is reduced, for example, short bowel syndrome (SBS), villous atrophy, enterocutaneous fistula, or proximal enterostomy. CEN has been associated with better feeding tolerance, nutrient absorption, and growth than boluses or oral feeds in infants and adult patients with intestinal disease [24, 25].

Indications

Indications for EN are different from indications for PN, since the use of EN as nutritional support is based on normal or at least partially preserved gut functions. The decision tree for the route of feeding is determined based on aspiration risk, motility function of the stomach, and anticipated duration of need for EN support. See Fig. 45.2. EN can be used on any patient with normal GI absorptive function but unable to adequately be fed by mouth. The conditions commonly encountered are listed in Table 45.1.

Conditions with Normal Intestinal Absorptive Function

EN is required in cases of inability to eat normally, that is, in those situations that are secondary to structural or functional abnormalities of the upper GI tract or neurological impairment of the processes involved in sucking and/or swallowing (see also Chap. 20). Esophageal diseases including esophageal atresia, fistula, or stenosis, often resulting from sequel of epidermolysis bullosa, are among the conditions that can benefit from EN, usually through gastrostomy or duodenal tubes [26, 27]. The choice of feeding through gastrostomy versus transpyloric tube must be assessed according to the patient age, disease, and condition (Fig. 45.2). Children with chronic diseases inducing immaturity or inability to feed orally, especially with sucking and swallowing troubles as seen in neurologically impaired children, with neuromuscular chronic diseases or cerebral palsy, also require EN, using GTs.

Premature Infants

EN via nasogastric or orogastric tube is routinely used in premature infants younger than 32 weeks' gestation because of uncoordinated suck, swallow, and breathing related to immaturity [28]. Human milk is the preferred feed because of its immunological benefits. Preterm infant formulas come fortified with protein, calcium/phosphorous, and a calorie density of 22–24 kcal/oz. to meet the high nutritional requirements of infants. A Cochrane systematic review of treatment trials did not provide evidence of any beneficial effect from transpyloric feeding over gastric feeding on feeding tolerance, growth, and development in preterm infants [29].

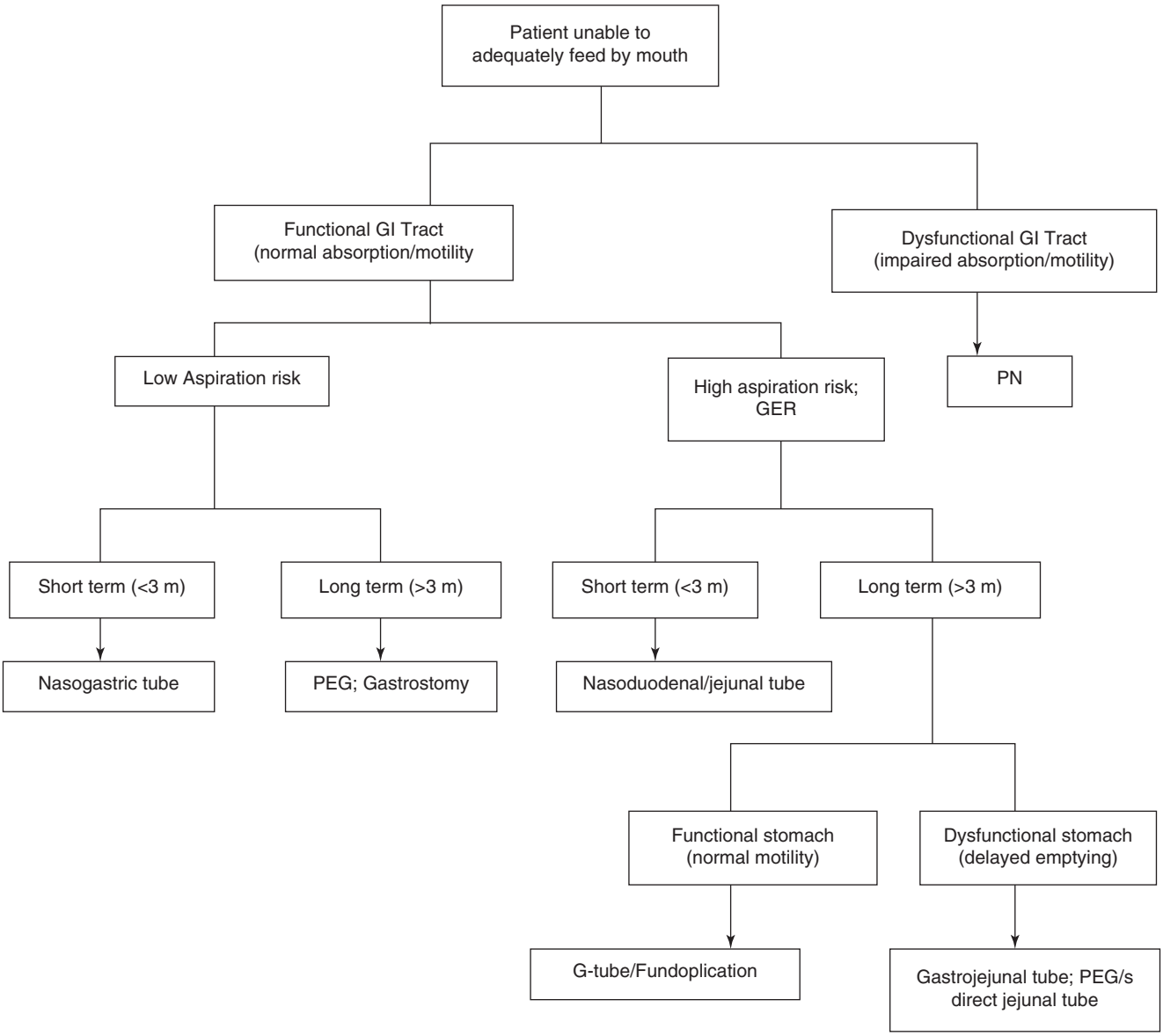

Fig. 45.2 Decision tree for the route of EN. *GER* gastroesophageal reflux, *PN* parenteral nutrition, GI gastrointestinal, PEG percutaneous endoscopic gastrostomy, PEG/J percutaneous endoscopic gastrostomy/jejunostomy, G-tube gastrostomy tube

Therefore, gastric feeding administered by bolus or continuously is the preferred approach for EN in preterm infants, where its use appears to also have a role in preventing necrotizing enterocolitis [30] (see also Chaps. 6 and 7).

Restrictive Eating Disorders: Anorexia Nervosa and Avoidant Restrictive Food Intake Disorder

Anorexia nervosa (AN) is a life-threatening psychiatric condition characterized by disordered eating behaviors, significantly lower than expected body weight, intense fear of becoming overweight, and a distorted body image. It is managed by a multidisciplinary team of health providers including psychiatrists, child and adolescent medicine pediatricians, nutritionists, and social workers. Indications for inpatient therapy include presence of suicidal or aggressive behaviors; severe bradycardia, hypotension, electrolyte imbalance, dehydration, and hypothermia; and medical complications, for example, seizures and pancreatitis [31]. Weight restoration is one of the major predicators for favorable short- and long-term outcomes in patients with AN [32]. Also, restoration of body weight is associated with improvements in malnutrition-induced cognitive impairments, thus facilitating psychological and psychiatric therapy [33].

Nutritional support in AN and avoidant resrictive food intake disorder (ARFID) remains very controversial. The

Table 45.1 Conditions commonly managed with EN

Conditions with normal intestinal absorptive function
Preterm infants
Critically ill patients
Nonorganic FTT
Anorexia
Inborn errors of metabolism
Glycogen storage diseases, urea cycle defects
Chylothorax
Hypermetabolic states
Head injury, graft versus host disease (GVHD), renal failure, congenital heart disease
Digestive disorders
Protracted diarrhea of infancy/childhood
Short bowel syndrome
Intestinal pseudo-obstruction
Crohn's disease
Malabsorption disorders
Cystic fibrosis
Chronic liver disease

FTT failure to thrive

oral feeding route is preferred; however, patients are given the option of voluntary or forced EN if resistant to therapy and/or severe malnutrition, that is, body mass index (BMI) < 13 kg/m^2 or z-score ≤−3 in adolescents [34, 35] and weight-for-height z score <−3 in younger children [36]. Other options like GT and PN are considered in young children or severe and chronic cases [31]. Follow-up of adults treated with EN during adolescence did not show any long-term benefits or adverse outcomes on growth, recovery, or persistence of AN or risk for development of psychiatric comorbidities [37]. The initial range of prescribed energy intake varies from 10–60 kcal/day to 1000 kcal/day and >1900 kcal/day with progressive increase during the course of hospitalization [31, 32]. The main complication of nutritional management is risk for developing re-feeding syndrome: hypophosphatemia, hypokalemia, edema, and increased hepatic transaminases. The risk factors associated with re-feeding syndrome include food intake <50% of estimated energy requirements for >1 month [38], greater severity of malnutrition, abnormal electrolytes prior to re-feeding, use of EN or PN, and weight loss >15% within the preceding 3 months [31, 32]. Patients may be preemptively treated with phosphate and thiamine supplements during the early phases of nutritional management.

Inborn Errors of Metabolism

EN is part of the standard therapy used to prevent biochemical abnormalities, metabolic decompensation, and catabolism in patients with inherited disorders of metabolism, for example, hepatic glycogen storage disease (GSD) and enzyme deficiencies of the urea cycle. Patients with GSD type 1 (glucose-6-phosphatase deficiency) develop hypoglycemia and compensatory biochemical abnormalities of lactic acidosis, hyperuricemia, hyperlipidemia, and platelet dysfunction all stemming from the primary defect of inability to dephosphorylate glucose-6-phposphate to free glucose. Managing GSD type 1 involves overnight continuous high-carbohydrate feedings and frequent daytime feedings supplemented with uncooked cornstarch [39, 40]. EN consisting of a glucose/glucose polymer solution or a sucrose-free, lactose-free/low formula enriched with maltodextrin may be used. EN should be started within 1 h after the last meal. Likewise, an oral or EN should be given within 15 min after discontinuation of the continuous EN because of the risk for hypoglycemia. Gastrostomy is contraindicated in patients with GSD type 1b because of complications in case development of inflammatory bowel disease and local infections [40]. Continuous EN should provide a glucose infusion rate of 7–9 mg/kg/min in children younger than 6 years, 5–6 mg/kg/min in children aged 6–12 years, and 5 mg/kg/min in adolescents [40]. Intermittent feedings of uncooked cornstarch may be used if continuous nighttime EN is not an option. No significant differences in biochemical parameters or growth have been found between patients with GSD type 1 receiving overnight continuous EN compared to scheduled feeds of uncooked cornstarch [41, 42]. The starting dose for uncooked cornstarch is 0.25 g/kg and optimal dose is 1.75–2.5 g/kg of ideal body weight every 6 h [40, 41, 43]. Patients with GSD type 3 (debrancher enzyme deficiency) have impeded glycogenolysis; however, gluconeogenesis is endogenously enhanced to maintain adequate glucose production. Therefore, nutritional management of patients with GSD type 3 involves frequent high-protein feedings during the day and a high-protein snack at night. Patients with GSD IV (brancher enzyme deficiency), GSD VI (phosphorylase deficiency), and GSD IX (phosphorylase kinase deficiency) respond to the high-protein diets similar to what is recommended for patients with GSD type 3 [44].

Inherited defects in urea synthesis are inborn errors of nitrogen detoxification and arginine synthesis due to defects in the urea cycle enzymes, namely, carbamoyl phosphate synthetase 1, ornithine transcarbamylase, argininosuccinate synthetase, argininosuccinate lyase, and arginase [45]. They may present at any age with symptoms ranging from poor feeding to coma shock and death. Other clinical symptoms may include lethargy, vomiting, ataxia, confusion, behavior changes, hypotonia or spasticity, hyperventilation (leading to respiratory alkalosis), and seizures. The main biochemical abnormalities are hyperammonemia and increased plasma concentrations of glutamine [46]. The symptoms of metabolic crisis are usually precipitated by protein intake in excess of what the patient can metabolize,

catabolism of lean body mass resulting from intercurrent infection, trauma, inadequate energy intake, or inadequate intake of protein or essential amino acids. Management of patients with urea cycle defects involves a combination of carefully restricting protein intake and therapy with nitrogen-scavenging drugs, for example, sodium benzoate, sodium phenyl acetate, or sodium phenyl butyrate [45]. The nutritional management includes (1) administration of sufficient energy to support anabolism, (2) restriction of protein intake to that tolerated by the patient without producing excess NH3, (3) provision of essential amino acids in adequate amounts to support growth, (4) supplementation of "conditionally" essential arginine or citrulline in all except arginase deficiency, and (5) provision of all required minerals and vitamins in amounts appropriate for age [47]. During hyperammonemic metabolic crisis, the nutritional management consists of providing high-energy low-protein intakes [48]. Successful long-term management requires a dedicated metabolic dietician and physician to make frequent dietary adjustments while closely monitoring progress. Patients with neurological handicaps or developmental delays, feeding difficulties, poor appetite/refusal of food, compliance problems with the diet, and/or medications will require use of nasogastric or gastrostomy feeding tubes to ensure adequate intake [45, 49]. Patients in metabolic crisis with symptomatic hyperammonemia (>500μM/L) and/or lack of response despite 3–6 hours of appropriate medical treatment should have management escalated to hemodialysis [45].

Hypermetabolic States

Hypermetabolic states include patients with burns, cancer, and head injury. Much of the morbidity and mortality in severely burned patients is connected to the prolonged hypermetabolism and catabolism, impaired wound healing, and sepsis. Whenever GI function permits, EN is superior to PN in patients with burns [50]. EN results in better regulation of the postburn catabolic hormones and inflammatory cytokine responses than PN [50, 51]. Furthermore, early EN support of patients with severe burns helps maintain gut mucosal integrity, which has the beneficial effect on reducing risk for gut-derived endotoxemia and infections [52].

Graft Versus Host Disease

Traditionally, PN is given as the first option for nutrition support in children undergoing chemotherapy and/or bone marrow/stem cell transplant. The reasons cited range from intestinal injury and poor GI tolerance secondary to conditioning and myeloablative therapy, intestinal graft versus host disease (GVHD), oral mucositis, epistaxis, and parental refusal of EN. However, whenever GI function permits, EN is equally as effective as PN, associated with lower risk for infection, and more cost-effective [53–56]. A prospective study comparing EN and PN in children with bone marrow transplants had poor enrolment into the EN group. Initiation of EN prior to transplant was associated with better overall tolerance. The EN group was also less likely to develop cholestasis [55]. A more recent Cochrane database review of nutrition support in patients of all ages with bone marrow or stem cell transplants failed to find evaluable data that properly compared efficacy and superiority of EN versus PN. However, the overall findings suggested that in patients without GI symptoms, intravenous fluids and oral diet should be considered as preference to PN [57].

Renal Failure

Supplemental nutrition should be given to children with renal failure to promote positive nitrogen balance and meet energy needs [4]. Children with chronic renal failure are at risk for malnutrition and growth retardation from persistent anorexia, inadequate protein calorie intake, chronic metabolic acidosis, azotemia, hormonal and metabolic disturbances, and catabolic diseases associated with uremia, for example, infections. Long-term EN is effective in preventing growth retardation in children with chronic renal disease and persistent anorexia, especially if started before the age of 2 years [58, 59], but singly may not lead to catch-up growth [60]. There is a positive correlation between efficacy of dialysis and linear growth of children with chronic renal failure [61]. Therefore, the combination of aggressive nutrition support with whey protein-based formulas in children age <2 years, whole protein enteral formula (1–1.5 kcal/mL) in older children [62], and enhanced dialysis is necessary for inducing growth in children with chronic renal failure [63]. Growth hormone therapy is recommended if there is persistent growth retardation despite adequate nutrition support [62, 64]. Ultimately, catch-up growth in height is mainly seen in children who undergo renal transplant before the age of 6 years [65].

Congenital Heart Disease

Inadequate calorie intake is the predominant cause of growth failure in infants and children with congenital heart disease. Different approaches for nutrition intervention are utilized during the preoperative, postoperative, and post-discharge periods, respectively [66]. Infants with cyanotic congenital heart disease and complex ventricular lesions are particularly susceptible to malnutrition and growth failure [67]. Approximately, 50% of infants with surgically treated univentricular lesions get discharged home on EN via nasogastric or GT [68].The causes of malnutrition include an imbalance between energy intake and expenditure, malabsorption, and/or end organ dysfunction due to impaired cardiac output. Infants with severe congenital heart disease have normal resting energy expenditure [69–71] but may have increased total energy expenditure [72], thus indicating that they expend large amounts of energy above basal requirements, which places them at risk for inadequate calorie intake when feeding at normal rates. Perioperative injury to the recurrent laryngeal nerve may lead to feeding difficulties from swallowing dysfunction and vocal cord paralysis [73]. Other contributing factors include pulmonary hypertension, tachypnea, and fatigue interfering with oral feeding; medications associated with anorexia, for example, diuretics; prescription of fat-restricted diets in patients who develop chylous effusions; and GI nutrient loss in patients who develop post-Fontan protein-losing enteropathy. Post-Fontan protein-losing enteropathy is managed with therapies directed at improving cardiac hemodynamics and controlling inflammation [74].

Digestive Indications

Since EN has a trophic effect on the intestinal mucosa and helps maintain mucosal integrity, it plays an important role in the treatment of many digestive diseases, either replacing or complementing oral feeding. Digestive diseases leading to an anatomical or functional reduction of the absorption capacity of the small bowel represent the first group; they include SBS, protracted diarrhea with villous atrophy, and inflammatory bowel disease.

Severe Protracted Diarrhea of Infancy/ Congenital Diarrheas and Enteropathies

A syndrome of intractable diarrhea of infancy was first described by Avery et al. in 1968. Its definition, presentation, and outcome have considerably changed during the past two decades [75]. This syndrome was defined as persistent diarrhea despite prolonged bowel rest requiring long-term total parenteral nutrition (TPN) in children when no effective treatment is available [76, 77]. Recent advances in understanding pathogenesis and increased availability of genetic testing have led to improved classification and characterization of many previously idiopathic chronic diarrhea illnesses of infancy [78]. Persistent and severe diarrhea presenting in the first weeks of life are now characterized as congenital diarrheas and enteropathies (CODEs). Most are monogenic diarrheal disorders that can be broadly classified into five major categories: (i) epithelial nutrient/electrolyte transport, e.g., congenital chloride diarrhea, congenital sodium diarrhea, glucose–galactose malabsorption, primary bile acid diarrhea, and acrodermatitis enteropathica; (ii) defects in epithelial enzymes and metabolism, e.g., congenital lactase deficiency, sucrase–isomaltase deficiency, trehalase deficiency, enterokinase deficiency, DGAT1 deficiency, PLVAP deficiency, abetalipoproteinemia, hypobetalipoproteinemia, chylomicron retention disease, dyskeratosis congenita, and Kabuki syndrome; (iii) epithelial tracking and polarity disorders, e.g., microvillus inclusion disease, tufting enteropathy, syndromic Na+ diarrhea, trichohepatoenteric syndromes 1 and 2, familial hemophagocytic lymphohistiocytosis 5, and TTC7A deficiency; (iv) enteroendocrine cell dysfunction, e.g., enteric anendocrinosis, x-linked lissencephaly and MR, proprotein convertase 1/3 deficiency, and Mitchell–Riley syndrome; and (v) immune dysregulation-associated enteropathy, e.g., IPEX, ICOS deficiency, ADAM17 deficiency, EGFR deficiency, CD55 deficiency, CTLA4 deficiency, LRBA deficiency, and XIAP. Algorithms are now available that improve evaluation, diagnosis, classification, and management of CODEs [78]. The CODEs resulting from defects in digestion, absorption, and transport of specific nutrients and electrolytes have normal villus and crypt architecture and thus may respond to dietary regulation restriction of the specific nutrients and electrolytes. Those with defects in enterocyte structure, epithelial cell defects, or abnormal villus to crypt ratios and morphology resulting from immune-mediated injury also have very poor tolerance of diet or EN and thus often need lifelong PN or allogenic intestinal transplantation [78]. Severely malnourished infants with some types of particularly severe celiac disease, intolerance to cow milk proteins, and protein hydrolysates or with specific malabsorption syndromes, such as Anderson's, disease may also benefit from CEN [79].

Short Bowel Syndrome

SBS is the leading cause of intestinal failure in newborns as well as infants and young children, and it is most commonly the result of an extensive intestinal resection during the neonatal period. EN is often used in these circumstances,

although several controversies over the ideal nutritional treatment of children with SBS remain [80]. EN is prerequisite for intestinal adaptation. EN exerts its trophic effects through direct contact with enterocytes and stimulation of local hormones. EN may also upregulate the expression of disaccharidases and peptidases in the colon of children with SBS, creating greater absorbtive capacity with less surface area [81]. The mechanism of this intestinal adaptation is believed to be in part due to changes in the microbiome, which are influenced by the presence or absence of an ileocecal valve [81]. PN therapy without any EN is associated with increased risk for intestinal failure-associated liver disease [82] (see also Chap. 43).

Disorders of Motility

In neonatal abdominal surgery for congenital or acquired disease, CEN, usually combined with PN, offers prolonged nutritional support which has transformed the prognosis in many conditions and is particularly important in the following situations: (1) reduction of the absorptive surface with enterocutaneous fistulae or extensive intestinal resection and (2) functional disorders of gut motility, such as malfunctions of duodenojejunal anastomosis, "plastic" peritonitis after repeated interventions, gastroschisis, and omphalocele.

Chronic intestinal pseudo-obstruction syndrome is a disabling disorder of enteral feeding characterized by repetitive episodes or continuous symptoms and signs of bowel obstruction, including radiographic documentation of dilated bowel with air–fluid levels, in the absence of fixed-lumen-occluding conditions [83]. Pseudo-obstruction is classified as "congenital" if newborn infants present with symptoms persisting for the first 2 months of life. "Acquired" pseudo-obstruction applies when previously well patients present with feeding intolerance from pseudo-obstruction symptoms persisting longer than 6 months [83]. Most patients with intestinal pseudo-obstruction require decompression stomas, ileostomies, and colostomies to relieve the recurring obstruction symptoms. Long-term PN is required in a significant proportion of children with pseudo-obstruction [84–86], and even then, administration of at least small volumes of CEN is essential and encouraged for prevention of PN-associated liver disease [87] and maintenance of bowel mucosal integrity [88].

Crohn's Disease

Enteral feeding has been used for many years, particularly in Europe, not only to improve nutritional status and growth but also to influence disease activity in patients with Crohn's disease (CD) [89–97]. The European Society for Parenteral and Enteral Nutrition (ESPEN) published updated guidelines in 2019 outlining a role for EN in inflammatory bowel disease not only in the context of providing nutritional support, especially before and after surgery, but also to induce remission and even as maintenance therapy [98]. It was shown that CEN diet is as effective as high-dose corticosteroids in inducing remission in pediatric patients with CD and has the added benefit of improved growth and development without the steroid side effects [99, 100]. However, serial meta-analysis conducted by Griffiths et al. in 1995, 2001, and 2007 suggested that therapy with EN was inferior to corticosteroids for inducing clinical response in patients with active CD [101–103]. All three analyses largely included adult studies, and several pediatric studies showing striking efficacy were excluded owing to methodological weakness. A more recent meta-analysis that focused on 11 randomized controlled pediatric studies still reported similar efficacy for EN and corticosteroids for inducing remission in CD but also cited limited data [104]. Two recent meta-analyses outlined not only that EN is as effective as corticosteroids in inducing remission but also highlighted mucosal healing as an advantage of EN over corticosteroids [105, 106]. The mucosal healing induced by EN is comparable to other first-line therapies, including infliximab, with the additional finding of fewer adverse effects in patients receiving EN compared to infliximab [107]. The benefits of EN appear to be more favorable in children than adults, including correction of impaired growth. The GROWTH CD Study, one of the largest multi-center prospective studies on this topic to date, further underscored the utility of EN in improving growth arrest, in addition to inducing remission, in pediatric patients with new-onset mild to moderate CD, even after correcting for confounders such as baseline severity and concomitant use of immunomodulators [108]. Thus, EN is an underused therapy with the advantage of preserving growth, while remission is achieved and therefore must be promoted [109]. There is no relevant data showing any significant difference in clinical outcome based on composition of the EN, that is, elemental, protein hydrolysates, or polymeric formula. Indeed, the beneficial effect in achieving remission appears not to be related to the mode of delivery or to the type of diet (no beneficial effect of elemental versus polymeric or semi-elemental) but rather to the reduced antigen load and mostly to changes in the intestinal microbiota [99]. The positive effects of EN also did not appear to differ if EN was administered orally or via CEN [99]. The Crohn's Disease Exclusion Diet (CDED) in combination with EN was shown to be a useful salvage regimen for patients who had poor response to anti-TNF therapy [110]. Removal of antigenic material, alteration in intestinal microflora, changes in gut

hormone levels, and the presence of bioactive transforming growth factor-β (TGFβ-1) in casein-based formulas may all play a role in the clinical success of EN [111, 112]. This change in the microbiota of patients receiving EN is believed to be the mechanism that leads to mucosal healing in these patients [113]. Glutamine-enriched polymeric diet offered no advantage over a standard low-glutamine polymeric diet in the treatment of active CD [114]. EN may be also helpful in correction or maintenance of the nutritional state, especially during a relapse of CD [100]. EN is part of the preparation for surgical procedure and may be useful during recovery, especially after intestinal resections or enterostomies. More on the treatment of Crohn's disease can be found in Chap. 28.

Other Malabsorption Syndromes

Cystic Fibrosis

Malnutrition and impaired growth affect approximately 23% of children with cystic fibrosis (CF) [115], and growth within the normal range of weight-for-length and BMI is associated with better pulmonary function and long-term survival. The CF Foundation recommends that growth assessment using percent ideal body weight (%IBW) be discontinued and instead rely on age-appropriate weight-for-length percentiles in children aged <2 years and BMI percentiles in children aged 2–20 years. The nutritional goals in children with CF are to maintain and support growth at weight-for-length or BMI at or above the 50th percentile [115]. Growth abnormalities in patients with CF have a multifactorial etiology including active CF-pulmonary or sinus disease, inadequate or ineffective pancreatic enzyme therapy, inadequate calorie intake, anorexia, presence of CF-related hepatobiliary disease, CF-related diabetes, infectious enteritis, small-bowel bacterial overgrowth, and other comorbid intestinal conditions including celiac disease and inflammatory bowel disease [116]. Whereas these disorders require specific therapy, nutrition monitoring and intervention in patients with CF should always be implemented regardless of comorbid disease. Improved weight status in patients with CF often requires energy and protein intakes ranging from 110% to 200% of normal needs in healthy people [116, 117]. However, even with an optimal approach to oral feeding, some patients respond poorly to nutritional counseling alone. Therefore, use of EN to supplement dietary intake is recommended to help restore and maintain nutritional status, especially in children aged 13 years and older who present with persistent growth deficits despite nutrition counseling [115]. The utilization of supplemental EN by patients with CF increased from 8.5% in 2001 to 11.1% in 2011 [118], thus indicating greater engagement of nutritional therapy. EN is generally performed during the night over 8–10 h with the initial goal of providing 30–50% of the estimated requirements [116, 119]. Patients with CF are then asked to eat and drink as much as possible during the daytime. Standard formulas containing intact protein, long-chain fat, and calorie densities of 1.5–2.0 kcal/mL are generally well tolerated with appropriate dosing and administration of pancreatic enzymes [116]. Semi-elemental formulas may be tolerated better in patients with excessive anorexia, bloating, or nausea. The data are unclear whether formulas with medium-chain triglycerides are advantageous over regular formula in patients with CF [116, 119].

Nasogastric tube (NGT) feeding is generally used as a first step. The tube is passed every night, 1–2 h after dinner, and removed in the early morning before physical therapy so that patients are not disturbed during the daytime for school attendance. In some children, NGT becomes increasingly uncomfortable because of nausea, vomiting, nasal discomfort as a result of nasal polyposis, or dislodgement during coughing in cases of pulmonary exacerbation. Gastrostomy tube (G-tube) is the preferred modality for administering home nocturnal EN when long-term EN is anticipated. Generally, G-tubes are perceived well by patients with CF who rely on EN therapy for poor weight gain or weight loss. On the contrary, patients and families without G-tubes were found to be apathetic toward their value, citing fear of interference with activities, embarrassment, pain, and discomfort [120]. Therefore, there is need for more accurate information about benefits and lifestyle in patients with G-tubes. Good early childhood nutritional status is associated with better pulmonary function and long-term survival in patients with CF [121]. However, poor growth and advanced lung disease have been associated with poor outcomes regardless of adherence to EN [122]. There is also currently insufficient evidence to determine whether nutritional rehabilitation after onset of malnutrition improves pulmonary function in patients with advanced CF-related lung disease, therefore the importance of a proactive approach of growth monitoring and early nutritional intervention to optimize growth in children and adolescents with CF. It is important to assess for gastroesophageal reflux before starting EN. Patients should also be monitored for glucose intolerance during administration of EN, especially during illness, therapy with steroids, and if not gaining weight. Insulin therapy should be administered as needed in patients with glucose intolerance [116, 119].

Chronic Liver Disease

Mechanisms leading to protein–energy malnutrition and requirement for EN in infants and children with chronic liver disease are multifactorial and dependent on the type of liver injury [123]. Malnutrition in cholestatic liver disorders is from reduced biliary secretion and intraluminal bile concentrations resulting in malabsorption of lipids and fat-soluble vitamins [124]. Protein and energy requirements are increased by different mechanisms including hypermetabolism [125], portosystemic shunting and ascites, futile metabolic pathways [126], and the energy demands from complications such as sepsis or variceal bleeding [127]. Liver disease from inborn errors of metabolism, for example, galactosemia, tyrosinemia, GSD, and Wilson's disease, requires specific dietary restrictions and EN feeding protocols to prevent hypoglycemia and other forms of metabolic decompensation. Patients with fulminant hepatic failure may be well nourished and nutritionally independent at clinical presentation but later require EN because of impaired mental status. Anorexia is common in children with chronic liver disease resulting from organomegaly, abdominal pressure effects of ascites, congested gastric mucosa, reduced motility from portal hypertension, central effects of unidentified toxins, dietary manipulations such as fluid restriction, or use of unpalatable feeds. Several factors contribute to long-chain polyunsaturated fatty acid (PUFA) deficiency including low PUFA intake, malabsorption, and disturbed metabolism of long-chain PUFAs. Finally, the interaction of growth hormone with insulin-like growth factor 1(IGF-1) and its binding proteins constitute an important mechanism linking nutrition and growth.

The most common cause of cirrhosis in children is biliary atresia, and the only definitive therapy is liver transplant [128]. A total of 60–80% of affected children are moderately to severely malnourished prior to transplantation [124], and poor nutritional status prior to transplantation is associated with prolonged hospital stay, increased risk for death, and high cost of medical care [127, 129, 130]. Supplemental EN or PN to improve nutritional status is the one intervention known to improve pre- and post-transplant growth and clinical outcomes in children with end-stage liver disease [131–134].

Anorexia, inadequate calorie intake, and failure to thrive are prevalent in children with chronic liver disease [124]; therefore, EN is recommended to supplement per oral food intake [127]. NGT feeding may be safely used without increased risk for bleeding in patients with portal hypertension varices [135, 136]. G-tubes should be avoided in children with chronic liver disease and portal hypertension because of likely portal-hypertensive gastropathy with increased bleeding risk from gastric varices [127]. MCTs are nutritionally advantageous in patients with cholestasis because bile is not required for digestion and they're rapidly absorbed into the portal circulation. MCTs may be administered separately as supplements; however, the selected EN should not contain more than 80% of fat as MCT because of the risk of inducing essential fatty acid deficiency [137]. Children with weight-for-length or body mass index z scores <−3 fall into the category of severe malnutrition, which is associated with a >9-fold risk for mortality [138], and therefore should be prioritized for nutritional intervention. Patients with chronic cholestatic liver disease have increased metabolism [125], futile metabolic pathways [126], and malabsorption; therefore, the goal for supplemental EN is to enable a daily calorie intake of 140–200% of estimated requirements [124]. Children with chronic cholestatic liver disease should continue receiving fat-soluble vitamins (A, D, E, and K) regardless of the amounts listed in EN [139]. Protein intake of infants and children should not be restricted except in cases of intractable encephalopathy [139], and, even in these cases, protein intake should remain within the adequate intake range of 1–2 g/kg/day [140] and accompanied by sufficient intake of non-protein calories to prevent inappropriate utilization of protein for energy synthesis [141]. Children with cholestatic liver disease have increased requirements for branched-chain amino acids (BCAA) compared to healthy controls [142]. However, nutritional outcome studies using either BCAA-enriched or standard formulas reported growth benefits in all recipients of EN [131, 132]. Furthermore, a Cochrane review did not find BCAA-enriched formula protective against encephalopathy when compared to iso-nitrogenous non-BCAA-enriched formulas [143]. Therefore, there is currently insufficient evidence to recommend the use of BCAA-enriched formulas over standard non-BCAA-enriched formula.

Chylothorax

Nutritional management of chylothorax involves adherence to EN or an oral diet enriched with medium-chain triglycerides, restriction of long-chain triglycerides, and supplementation with essential fatty acids and fat-soluble vitamins [144]. Nutrition support during the preoperative period utilizes EN in infants who are hemodynamically stable and early use of PN if otherwise. The immediate postoperative period is characterized by fluid restrictions and hemodynamic instability and therefore requires active involvement of a dietician and reliance on PN to meet goal calories. Once hemodynamic stability is established, enteral feeding is introduced while following standardized protocols that include screening for swallowing dysfunction and management of GI symptoms, for example, reflux. Nutrition surveillance should be performed continually pre- and post-discharge

to guide further interventions including use of calorie-dense formula and home tube feeds [66].

Techniques of Delivering Enteral Nutrition

The route of EN administration should be individually tailored, depending on the underlying condition (Fig. 45.1). Enteral feeding is preferable to PN, and therefore it should be immediately implemented in children with functional GI but unable to feed per oral. The absolute contraindication to EN includes mechanical obstruction of the GI tract (unless indicated for decompression), active peritonitis, uncorrectable coagulopathy due to increased risk of bleeding, or bowel ischemia [145, 146]. The intragastric route of administration is the most commonly used in children, since it is the more physiologic route which permits the action of salivary and gastric enzymes, the bactericidal action of gastric acid, and the better mixing with biliary and pancreatic secretions. Therefore, the duodenal or jejunal route is used in very few circumstances in children. For intragastric administration EN, NGT, or GT may be used. NGT feeding is the best initial approach to EN, to evaluate the tolerance of EN before placing a permanent GT, and/or when a brief period of EN support is anticipated.

NGT is made of polyvinylchloride (PVC), polyurethane, or silicone. Modern feeding tubes are made of either silicone or polyurethane [147]. Polyurethane tubes come externally and internally impregnated with a water-activated lubricant to ease insertion through the nasopharynx and facilitate removal of introducer wires [148]. They are also more resistant to degradation and deterioration when compared to silicone tubes. Tube size (outer diameter) is described in French gauge (Fr) units. The millimeter conversion can be derived by dividing each Fr by π (3.14). The length of insertion for a nasal- or oral–gastric feeding tube to be in a child is determined by using either the morphological markers of "nose–ear–mid-xiphoid–umbilicus" span or age-specific prediction equations. Length predictions based on just a "nose–ear–xiphoid" span are likely to result in a placement that is too proximal [149]. At the time of NGT placement, aspirating fluid and measuring a pH $\leq$ 5 are the most reliable bedside tests confirming gastric placement in children [150]. However, the usefulness of this test may be limited in patients being treated with gastric acid suppressants. Simple auscultation is not a reliable method for assessing position because injection of air into the tracheobronchial tree or pleural space can produce an indistinguishable sound. Therefore, an abdominal X-ray is the gold standard for establishing location of the NGT tip [150].

Duodenal or jejunal tube placement is more difficult; the patient should be placed in the right lateral position and, if necessary, after an intravenous injection of erythromycin. The position of the distal end of the tube is then checked by abdominal X-ray. Careful nasal fixation of the tube is used to avoid displacement; it is taped to the upper lip, the ipsilateral cheek, and the external ear. In some particular indications for EN, the feeding tube may also be introduced through the mouth, especially in patients with congenital or acquired nasal obstruction and premature infants on respiratory therapy with continuous positive air pressure (CPAP). The placement of NGT made of PVC is easier, but these tubes should be changed more frequently as they become more rigid. Silicone or polyurethane NGT may be used over 3-week periods or more. However, silicone and polyurethane are generally more flexible and easily displaced by vomiting. They are preferentially used for transpyloric and long-term EN. Individualized goals for growth, nutrition, and when to discontinue EN should be established prior to insertion of a feeding tube. Use of EN may only be temporary in acutely ill but previously healthy children, or as a long-term source of supplemental nutrition in children with chronic inadequate per oral food intake, and the sole source of nutrition in children with severe disability in per oral feeding.

When a child's duration on EN is longer than 30 days and feeds well tolerated yet low anticipation for timely acquisition of adequate per oral feeding skills, transition to a feeding gastrostomy should be considered [146, 147]. Percutaneous approach to placement of gastrostomy has revolutionized the placement of enteric feeding tubes in children. The standard approach involves use of a gastroscope for gastric transillumination and stoma site identification. This is followed by percutaneous introduction of a guide wire that gets grasped using the gastroscope and then orally withdrawn. Thereafter, the gastrostomy catheter is attached to the oral end of the guide wire and then pulled in an antegrade manner through the oropharynx, esophagus, and stomach and then across the gastric and abdominal walls [151]. It may be placed using GI endoscopy and surgical laparoscopy or by interventional radiology [147]. Following placement, postoperative care mostly involves pain management, and most providers permit the use of GT within 12–24 h. The initial PEG tube is changed after 2–3 months, by which time a good tract has formed. Button-replacement gastrostomy devices provide patients a cosmetic advantage in the case of long-term EN.

Percutaneous placement of gastrostomy or jejunostomy is contraindicated in patients with previous abdominal surgery, abnormal abdominal anatomy, or severe deformities of the chest and spine which modify the position of the stomach and other intra-abdominal viscera. In such cases, a surgical GT should be placed. The implantation of a jejunal feeding tube, via G-tube (gastrojejunal tube), is a possible method for the treatment of inadequate oral feeding in patients who are affected by gastroesophageal reflux (GER), gastroparesis, and increased risk for aspiration and is thus an alternative to fundoplication and prokinetic medications [151, 152]. However, gastrojejunal feeding tubes are also prone to technical complications requiring replacement because of clog-

ging and recoil of the jejunal catheter back into the stomach [152]. EN may be delivered as boluses or prolonged continuous feeding using a feeding pump, syringe, or controlled delivery by gravity. Pumps recommended for pediatric use have to provide clear flow rate display and alarms. Miniaturized and battery-powered pumps are specially designed for home and ambulatory EN.

Nutrients

Nitrogen

The absorption of amino acids is more rapid and efficient when given in the form of short peptides than free amino acids [153, 154]. Therefore, in order to maximize nitrogen assimilation in patients with marked impairment of gut absorptive capacity, the ideal EN should consist of di- and tripeptides and free amino acids [154]. In addition, the quality, in terms of digestion and intestinal absorption of protein hydrolysates, depends on the type of hydrolysate, for example, lactalbumin is superior to casein [155]. However, in patients with normal GI function, EN with formulas consisting of protein hydrolysates offers no nutritional or absorptive advantage over EN based on formula consisting of free amino acids or intact protein [156]. Thus, the initial formula in patients with normal GI function should be based on intact protein or polypeptides, with a lower osmolality, rather than on a mixture of free amino acids and short polypeptides.

Carbohydrates

Disaccharidase enzymatic activities are depressed in disease involving the small intestine mucosa. Lactase appears to be the most sensitive to injury and the last of the disaccharidases to recover. In addition, certain drugs such as neomycin or colchicine depress the intestinal disaccharidases. Thus, it is important to avoid dietary sources of lactose. Other disaccharides should also be omitted from the solution used for initial feeding as their corresponding brush border enzymatic activities are reduced. The carbohydrate source allowed during the EN in patients with normal GI function can be lactose. However, patients with impaired GI function should be fed an EN containing lacto-free glucose polymer as the carbohydrate source.

Lipids

The main dietary lipids are triglycerides structurally made up of three fatty acids linked to a glycerol molecule. The fatty acids contain between 2 and 24 carbon atoms (C: 2 and C: 24). Classification of the triglycerides is based on length of the fatty acid chain, that is, short-chain triglycerides (SCT) contain fatty acids that range in length from 2 to 4 carbons (C: 2 to C: 4); the MCTs contain fatty acid chains ranging from 6 to 12 carbons (C: 6 to C: 12); and LCTs have >12 carbons. In contrast to the LCTs, dietary MCTs are hydrophilic and do not require bile salts or micelle formation, and their free fatty acids are directly absorbed into the portal system without requirement for re-esterification into chylomicrons [14, 157]. MCTs are hydrolyzed faster than LCTs in the small intestine by pancreatic lipase; they are converted almost exclusively into free fatty acids and glycerol and reach directly the portal circulation and the liver. Nevertheless, in the case of pancreatic insufficiency, MCTs may also be absorbed intact. The excessive use of MCT-containing diet can lead to osmotic diarrhea as a result of their rapid hydrolysis. Dicarboxylic aciduria has been described in infants supplemented with MCT-rich formulas without any proof of deleterious effect [158]. The provision of essential fatty acids (EFA) must be considered since MCTs contain no EFA. Furthermore, MCTs decrease LCT absorption; thus, supplementation with linoleic acid is necessary. However, its addition to a formula based on MCTs may be insufficient to prevent EFA deficiency, thus making it necessary to provide EFA parenterally. Nevertheless, most formulas containing MCTs also include up to 50% of lipid as LCTs. LCTs in excess in the intestinal lumen, especially if they are hydroxylated by bacteria, reverse the rate of water and electrolyte absorption and increase malabsorption. Finally, a lipid intake of 3–4 g/kg/day may be achieved, depending on absorption capacity and digestive tolerance.

Other Components

Recommendations concerning energy, water, and electrolyte supplies in premature and full-term infants are provided in the chapter on PN in the premature infant. In older children, the recommended energy intake is based on recommended daily intake (RDI) values or may be estimated using the World Health Organization (WHO) weight, age, and gender prediction equations or directly measured using an indirect calorimeter [140]. The minimum daily fluid requirements with some exceptions may be estimated using the "Holliday–Segar" calculation, which is an extrapolation based on daily calorie expenditure. For weights ranging from 0 to 10 kg, the estimation is 100 mL/kg/d; from 10 to 20 kg, the estimation is 1000 mL plus 50 mL/kg for each kilogram of body weight more than 10; and over 20 kg, the estimation is 1500 mL plus 20 mL/kg for each kilogram more than 20 [159].

Choice of a Formula

Enteral feeding formulas are divided into several families. The choice of a formula is made according to numerous parameters like protein–calorie needs, digestive function,

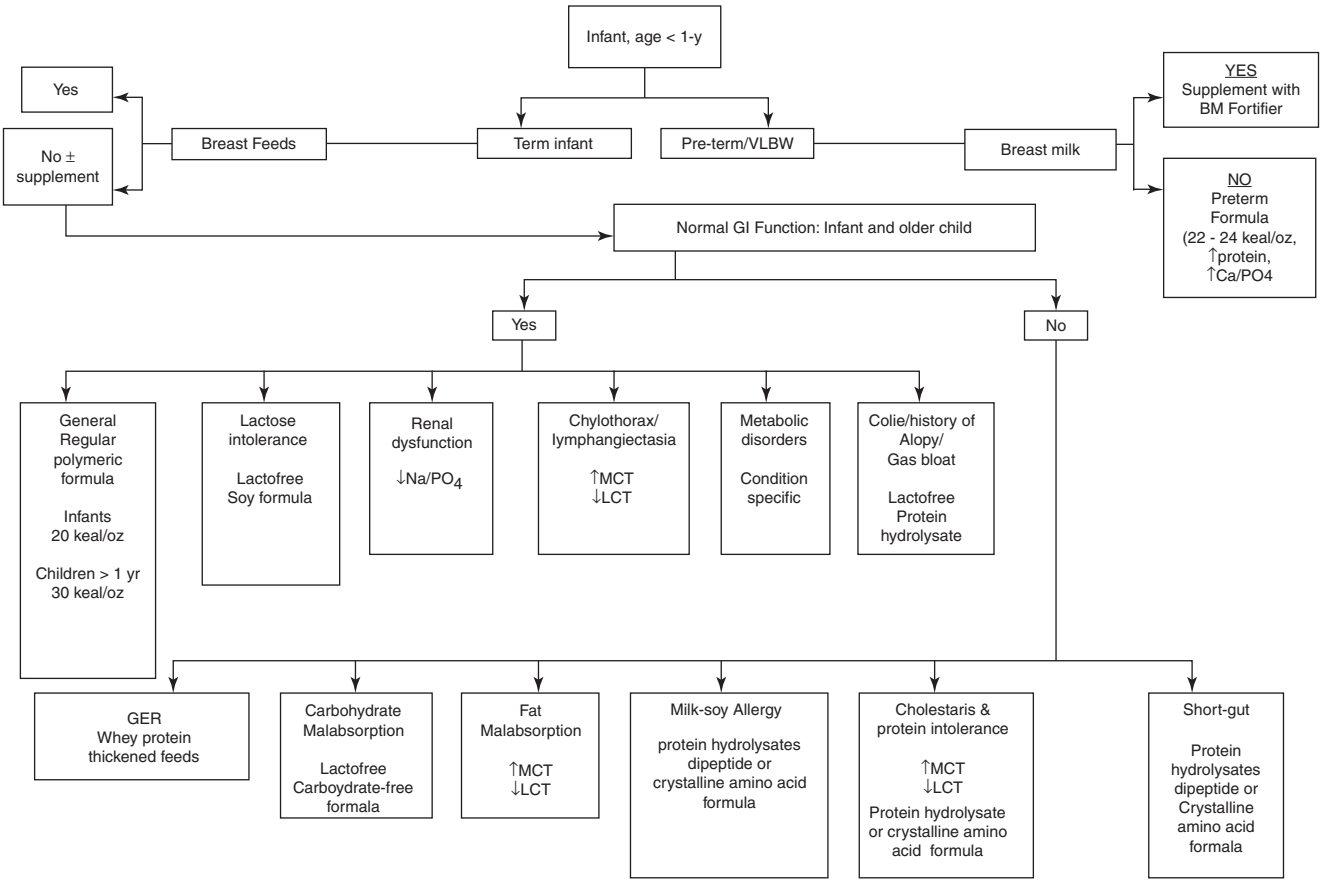

Fig. 45.3 Algorithm for selection of formula. GER gastroesophageal reflux, MCT medium-chain triglycerides, LCT long-chain triglycerides, EN enteral nutrition

protein sensitivity, motility status, and tolerance to fluid intake, all obviously dependent on the age and on the underlying disease (Fig. 45.3). In preterm, newborns, and young infants with normal intestinal function, human milk or standard infants formulas supplemented with long-chain polyunsaturated fatty acids (LCPUFA) may be used. Formulas for preterm infants are unique in being more calorically dense (72–90 kcal/100 mL) with increased protein (1.8–2.3 g/100 mL), calcium (70–108 mg/100 mL), and phosphorus [160]. The preterm formulas are continued after hospital discharge in preterm or small for gestation-age infants when discharge growth parameters are below appropriate post-conception growth, and continued until achievement of catch-up growth [160]. Breast milk and standard infant formulas have a calorie density of 0.67 kcal/mL with lactose as the carbohydrate source and fat source compromised of LCTs and LCPUFA (DHA and EPA). Commercial polymeric formulas are available for older children (age >1 year), and blenderized diets can be prepared using food from the kitchen and may be used in the nonstressed patients with normal gut function. The calorie density of formulas used in older children (age >1 year) ranges from 1 kcal/mL (standard) to 1.5–2.0 kcal/mL (calorically dense).

In the case of GI disease, the choice of the nutritive solution must take into account not only the child's age and nutritional status but also the underlying digestive disease, for example, the presence of anatomical and functional changes in the intestine, whether due to an extensive reduction of the absorptive surface, to enteropathy, or to pancreatic insufficiency. Limiting factors in such cases are impairment of gastric, biliary, and pancreatic secretions, disturbances of the intestinal flora, and malabsorption. Most standard (1 kcal/mL) polymeric formulas have osmolality within the range of 300–360 mosmol/kg. Oligomeric (hydrolysate/semi-elemental) and elemental (crystalline amino acid based) enteral formulas are designed for use in patients with malabsorption such as in short bowel syndrome, CF, cholestasis, food allergy, or intolerances. These diets include dipeptides, tripeptides, and a few free amino acids, combined with LCTs and MCTs, and carbohydrates including glucose polymers and maltodextrins.

The osmolality of a solution is determined by the number of particles within it. Likewise, the osmolality of enteral formulas is directly influenced by the calorie density and whether it is made up of intact protein, peptides, or amino acids. Therefore, free amino-acid-based formulas tend to have a

higher osmolality. Children with severe malabsorption or short bowel syndrome unable to tolerate peptide-based formulas (protein hydrolysates) might benefit from the free amino-acid-based formulas [161, 162]. Nutritional formulas in which each of the constituents is modified independently are mostly used in special conditions, for example, selective malabsorption. In that case, glucose may be given initially as calorie source, the amount being increased progressively and controlled according to the stool volume, pH, and absence of reducing substances in the stools. In the first days of feeding, at least a molar ratio of glucose and sodium is maintained.

Regulation of Intakes and Rhythm of EN Delivery

EN should be progressively introduced depending on the child's nutritional status and the indications for EN. In the case of digestive disease, CEN can be used after a brief or prolonged period of PN. The first step includes the progressive reduction of the parenteral intake and the stepwise increase of EN according to the digestive tolerance.

The rhythm of EN delivery depends on the underlying disease. Intermittent feeding using bolus is more physiological and well tolerated when the digestive function is normal. Continuous cyclic nocturnal EN is better tolerated in some patients who do not tolerate bolus feeding and provides less interference with daytime oral intake. On the other hand, continuous 24/24 h rhythm of delivery is indicated in the case of impaired digestive function. The weaning period varies from few days to several weeks or months. Eating disorders can be avoided by the maintenance of sucking and swallowing functions during the period of CEN. On the other hand, it has been demonstrated that nonnutritive sucking intervention during CEN in preterm infants resulted in faster transition from feeding tube to oral feeds, better bottle feeding performance, enhanced growth and intestinal maturation, and decreased duration of hospital stay compared to preterm infants without the intervention [163, 164]. In older children, weaning from EN may include a period of continuous nighttime feeding supplemented by several meals in the daytime until the latter account for 50% of the total intake. Oral feeding must be carefully increased because of the relatively low intestinal activity due to long-term CEN.

Complications of Enteral Nutrition Therapy

Although complications occur rarely, they can be quite serious. Strict adherence to the procedure is indicated and careful supervision is essential to prevent them. See Table 45.2.

Table 45.2 Complications of enteral feeding tubes and therapy

Nasogastric	PEG/gastrostomy
Complications during tube insertion	
Arrhythmias	Aspiration
Pyriform sinus perforation	Hemorrhage
Esophagus perforation	Peritonitis
Tube in pulmonary tree/pulmonary intubation	Necrotizing fasciitis (rare)
Pneumothorax	Ileus
Empyema	Fistulous tracts
Gastric perforation	Perforation of viscera
Duodenal perforation	–
Complications when in situ	
Otitis media	Peristomal infection
Sinusitis	Stomal leakage
Epistaxis	Buried bumper
Nasal mucosal ulceration	Gastric ulcer
Pulmonary aspiration	Inadvertent removal
Gastroesophageal reflux	–
Tube dislodgement	–
Functional complications	
Diarrhea	
Clogging	
Knotted tubes	
Contaminated feeds: *Cronobacter* spp. (formally *Enterobacter sakazakii*), *E. cloacae*	
Re-feeding syndrome	
Feeding aversions	

Functional Complications of Feeding Tubes

The functional complications of enteral feeding tubes include tendency to clog, which occurs 18–45% of the time [165]. The risk is higher in smaller-diameter tubes, and the cause of occlusion includes interaction of protein-based formulas with an acidic environment and medications, and complete obstruction from knotting of the feeding tube [165–167]. Clogged NGTs should be readily replaced without the need to apply extraneous efforts to unclog. However, a good effort should be made to unclog blocked nasojejunal and gastrojejunal tubes because they are more difficult to insert. Clogging and occlusion can be minimized by frequently flushing the tube before and after administration of feeds. Water is more effective than carbonated beverages for flushing and unclogging occlusions. Sterile water is preferred for flushing because several published cases of infection were traced to tap water [145]. Persistently clogged tubes despite water flushes may respond to installation of one crushed tablet of Viokase (pancreatic enzyme) in 5 mL water and pH raised to 7.9 using NaOH instilled into the tube using a 50 mL syringe under manual pressure for 1 min, then clamping the tube for 5 min [167]. Other options include trial of meat tenderizer or use of mechanical devices such as Fogarty balloon and biopsy brush. Failure to unclog the tube requires replacement of the tube [145].

Gastrointestinal Complications

Risks and complications start with passage of NGTs, which can be hazardous in any patient, especially those with poor or impaired gag reflex, absent cough reflex, or altered consciousness. Furthermore, presence of a cuffed endotracheal tube is not guaranteed protection against pulmonary intubation. Therefore, feeding should not begin until proper placement of the tube is verified [146]. The bedside assessment tools of visually inspecting for color and testing for pH of aspirates may be inaccurate because small bore tubes may collapse resulting in failure to drawback aspirates, and therapy with gastric acid suppressants may affect the measured pH. The auscultation method is also problematic because sounds may be transmitted to the epigastrium regardless of tube placement in the lung, esophagus, or stomach. Some protocols call for the first feed to be water to ensure tolerance [146]. Overall, abdominal X-ray is the gold standard for establishing location of the NGT tip [150].

Aspiration pneumonia is the most threatening complication associated with NGT feeding [146]. Irregular flow rate of infusion, delayed gastric emptying due to the underlying disease or to medications, GER, tube placement or migration into the distal esophagus, behavioral vomiting, and formula intolerance are risk factors for vomiting and aspiration. The general prevalence of feeding-tube-related aspiration pneumonia is unknown; however, the incidence in critically ill patients ranges from 25% to 40% [163] with both oropharyngeal and gastropharyngeal contents implicated. The risk factors in critically ill patients include decreased level of consciousness, vomiting, malpositioned feeding tube, larger-diameter NGT, and bolus feeding. The preventative measures include keeping the patient in a semi-recumbent position, continuous aspiration of subglottic secretions, and change from bolus/intermittent to continuous EN [168, 169]. Tubes placed past the third portion of the duodenum are associated with decreased risk of aspiration [146].

Diarrhea is the most common complication of EN and is reported in up to 68% of patients [146]. Increased stool losses occur when the combined absorptive capacity of the small bowel and the salvaging capacity of the colon are exceeded (see Ref. [170] for a review of the role of the colon in short bowel syndrome) and may result in dehydration and hypoglycemia. The causes include inappropriate composition of the formula for the underlying disease, high formula osmolality and rate of infusion, intraduodenal infusion, hypoalbuminemia, and bacterial contamination of the formula. The volume of endogenous fluid flux into the jejunum is directly proportional to the osmolality and rate of infusion of the EN [171]. Therefore, since higher osmolality (>300 mosmol/kg) feeds will induce greater endogenous fluid flux into the bowel, their rates of infusion should be increased cautiously.

Mechanical, Infectious, and Metabolic Complications

NGT-related mechanical complications include nasal trauma, laryngeal ulceration, or stenosis, and esophageal or gastric perforations. Duodenal perforations have been reported in association with transpyloric feeding tubes in preterm infants, neonates, and critically ill children regardless of whether they are polyvinyl, silicone, or polyurethane feeding tubes [172–174]. In one series of 526 critically ill children receiving transpyloric EN, the prevalence of GI complications was 11.5%. These included abdominal distension and/or excessive gastric residue (6.2%), diarrhea (6.4%), GI bleeding, necrotizing enterocolitis, and duodenal perforation (0.9%). The major factors associated with risk for developing these complications were shock, epinephrine dose >0.3μg/kg/min, and hypophosphatemia [175]. Therefore, frequent re-evaluation and a high index of suspicion are required in this patient population. Complications from PEG placement may occur during the immediate postoperative period and/or delayed for several days. The postoperative complications include aspiration, hemorrhage, peritonitis, necrotizing fasciitis, peristomal infections, prolonged ileus, fistulous tracts, and inadvertent removal. The delayed complications include site infections, persistent peristomal leakage/irritation, buried bumper syndrome, gastric ulcer, fistulous tracts, inadvertent tube removal, and fungal tube infections, especially affecting silicone tubes leading to tube degradation and malfunction [145]. Children without fundoplication may go through a period of worsened reflux symptoms [176–178] that may respond to slower advancement of EN or necessitate a brief period of change to continuous feeding schedule.

Patients with PEGs placed too close to the pylorus, and 24–30% of children with fundoplication, may develop metabolic dumping characterized by postprandial tachycardia, diaphoresis, lethargy, refusal to eat, gas bloat, and water diarrhea in association with bolus feeds [179–181]. Establishing a screening protocol for postprandial hypoglycemia in patients with history of fundoplication is important because approximately 46% of affected children fail to exhibit symptoms [181]. The diagnosis of dumping is confirmed by a glucose tolerance test showing postprandial hypoglycemia preceded by postprandial hyperglycemia. The management options include initial avoidance of boluses, change to continuous feeding schedule, modification of the EN to avoid

lactose, change to a complex carbohydrate source, supplementation of bolus feedings with uncooked cornstarch [182–185], and in refractory cases, therapy with ascorbate (disaccharidase inhibitor) [186].

Infectious Complications

EN has been associated with outbreaks of antimicrobial-resistant organisms. *Cronobacter* sp. (formally *Enterobacter sakazakii*) is a rare but one of the most important worldwide causes of outbreaks of neonatal sepsis and meningitis associated with non-sterile powdered infant formula or human milk fortifier, and has a mortality of 40% [187]. Ninety-nine percent of affected patients were infants aged <2 months. The infection was acquired by infants in hospital or at home, and the predominant risk factor was the use of powdered infant formula or human milk fortifier [187, 188]. Therefore, the US Food and Drug Administration has issued the recommendation that the use of powdered infant formulas and human milk fortifiers should be minimized in hospitalized preterm or immunocompromised neonates [189]. Ready-to-use commercial formulas are sterile and therefore recommended for EN in hospitals.

Other sources of organisms include post-manufacturer microbial contamination exogenously introduced during manipulation of the formula and through retrograde contamination of the feeding tube system by the patient's endogenous flora. The enteral feed administration sets become colonized externally by microbes grown from the enteral tube hub and may serve as a reservoir of organisms that can be cross transmitted [190–192]. Commercial liquid formulas received from the manufacturer are required by regulatory agencies to be shelf-stable and free of enteric pathogens. The more common sources of organisms are usually exogenous the patient acquired through contamination of locally prepared or post-manufacturer manipulation of ready-to-use formula [190]. Microbial contamination of the enteral tube hub may result from retrograde migration of endogenous bacteria along the tubing system [193], following which nosocomial cross-transmission to other patients may occur when care providers handling the enteral tube hub fail to practice strict infection control procedure [194, 195]. Therefore, adherence to standard precautions is critical when handling enteral feeding apparatuses. The bacterial contamination of the nutritive solution with enterotoxin-producing bacteria like coliforms or *E. cloacae* [192], at the time of preparation, storage, or delivery to the patient, has been associated with gastroenteritis and/or septicemia [191, 196]. The relationship between bacterial contamination of enteral formula and diarrhea may be a matter of debate. When EN is delivered into the stomach, there is a protective effect from gastric acid. Therefore, the risk of bowel contamination is theoretically higher when EN is delivered distal to the pylorus. Since necrotizing enterocolitis (NEC) may occur in premature infants and neonates suffering from hypoxia and infections, the abdomen must be checked daily very carefully. Because of the risk of infectious complications and NEC, using gastric route, avoidance of duodenal infusions, and utilization of ready-to-use preterm formulas are recommended in preterm infants.

Re-feeding Syndrome

This is a metabolic syndrome induced by feeding that occurs in previously starved or chronically malnourished patients. The syndrome is characterized by hypophosphatemia, hypokalemia, hypomagnesemia, arrhythmia, edema, congestive heart failure, and increased risk for death. The incidence of re-feeding syndrome in children is not known; however, it is reported in up to 50% of hospitalized adults documented to be malnourished [197]. It results from rapid anabolism induced by increased insulin secretion in response to provision of nutrients. The increased glucose load, with corresponding increase in the release of insulin, leads to cellular uptake of glucose, potassium, magnesium, and phosphate. This rapid shift in electrolytes from the extracellular to intracellular space results in hypokalemia, hypomagnesemia, and hypophosphatemia. Insulin also exerts a natriuretic effect on the kidneys resulting in sodium and fluid retention. The anabolism may unveil or cause other nutrient deficiencies, for example, thiamine deficiency and beriberi, further leading to life-threatening circumstances [198]. The pre-nutrition therapy determinants of risk for developing re-feeding syndrome include patients underfed or not fed for at least 10–14 days (regardless of therapy with crystalloid intravenous fluids), acute weight loss of >10% in the preceding 1–2 months, severe malnutrition as defined by <80% ideal body weight or weight-for-age z-score <−3 [36], decreased pre-albumin [199], anorexia, marasmus, edematous protein–energy malnutrition (kwashiorkor), children of neglect, cerebral palsy, and other conditions causing dysphagia [198]. The risk for developing re-feeding syndrome is higher during the first 5–7 days of implementing nutritional therapy. The metabolic derangements can be avoided by initiating feeds at a calorie intake of 50–75% of the measured or estimated resting energy expenditure with appropriate supplementation of phosphorus. The calorie intake is then increased by 10–20% per day with close monitoring and correction of biochemical parameters until the calorie goal is met [200].

Home Enteral Nutrition (HEN)

Indications

HEN is a logical alternative to long-term hospitalization when a long-term enteral nutritional support is necessary (more than 1 month) in a patient in stable clinical condition. HEN has been proven to be an effective and safe method, compatible with the best possible quality of life [201–203]. Major cost-savings induced by home EN as compared to hospitalization have been demonstrated. The importance of families' teaching and medical follow-up to prevent somatic and psychological complications should not be underestimated [76].

Organization

The quality of home EN programs depends on the organization of multidisciplinary nutrition support teams based on a tight collaboration between the different professionals including physicians, home care nurses, dieticians, pharmacists, and social workers. In some countries, pumps, disposable equipment, and nutrients for HEN are mainly delivered by hospitals, while in others, such as in the USA where they are the most developed, home care companies participate to patients' training and provide a delivery service and a 24 h emergency phone contact. With expansion of the home care industry, it is important to ensure that adequate standards of care are provided. The American Society for Parenteral and Enteral Nutrition and the British Association for Parenteral and Enteral Nutrition have produced such standards [4, 204].

Parents' Teaching

Tolerance and efficacy of EN should first be assessed in the hospital before transitioning to HEN. As for HPN, HEN in children is feasible only when the family is highly motivated and able to deal with technical aspects. Parent's teaching is based on a nutrition multidisciplinary hospital team including physician, nurse, dietician, and pharmacist. Parents are taught not only about technical aspects but also about risks, complications, and their prevention. The hospital team ensures a 24/24 h phone contact and regular follow-up, in tight cooperation with the general practitioner. The help of a community nurse may be required, especially in the case of placement of a nasogastric tube.

Results

Quality of Life

Although HEN, like HPN, is usually considered by children and families as an improvement of their quality of life, psychological consequences of this technique have to be carefully estimated and prevented. The placement of a NGT is distressing to parents and children. A NGT in situ for continuous 24/24 h infusion is a major problem, particularly in oldest children and adolescents, because it induces an unwelcome public interest. These problems are solved by the use of gastrostomy. Children and families may also suffer from the suppression of meals taken together, which are usually considered as an important moment of the family life. Adequate psychological preparation and follow-up improve tolerance of children and parents to HEN [205, 206].

Outcomes

Outcome studies on HEN patients have been fewer than those on patients on HPN. Improvement of the nutritional status and low mortality rate (always related to major underlying diseases) are usually described in children on HEN [207].

Cost and Funding

HEN is far from being as expensive as HPN. The total cost per day of HEN was about $35 in the USA in 1992, when the cost per day of HPN was about $300 [208]. The cost-savings associated with providing HEN estimated by the British Association for Parenteral and Enteral Nutrition in 1994 were about 70% [204]. In most countries, patients on HEN are funded by the National Health Service, although in the USA they are mostly paid for by insurance companies [204, 209].

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Parenteral Nutrition in Infants and Children

46

Susan Hill

Abbreviations

CSPEN	Chinese Society of Parenteral and Enteral Nutrition
CVC	Central venous catheter
EFA	Essential fatty acids
EN	Enteral nutrition
ESPEN	European Society for Clinical Nutrition and Metabolism
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
ESPR	European Society for Paediatric Research
IF	Intestinal failure
IFALD	Intestinal failure-associated liver disease
MDT	Multidisciplinary team
MUAC	Mid-upper arm circumference
NST	Nutrition support team
PDD	Primary digestive disorder
PICC	Peripherally inserted central venous catheter
PN	Parenteral nutrition
PNDD	Primary non-digestive disorders
SBS	Short bowel syndrome

Introduction

Parenteral nutrition (PN) can be defined as the infusion of nutrients directly into the bloodstream, by passing the gastrointestinal tract. PN is a lifesaving supportive treatment in intestinal failure (IF) that should be considered one of the major medical advances of the last century. The use of PN has enabled even the sickest child, including the most premature of infants, to survive whilst the underlying disease is diagnosed and treated. With improvement in PN and neonatal intensive care outcome, the number of infants and children surviving with IF has greatly increased over the last 15 years [1].

IF has been defined as the inability to absorb sufficient fluid and nutrients from the intestine to maintain homeostasis and normal growth and development even when the most suitable enteral nutrition (EN) is given via the most appropriate artificial feeding device for the patient [2]. The three major aetiologies of chronic IF are short bowel syndrome (SBS), intestinal motility disorders and small intestinal mucosal disease.

PN treatment is used not only for IF with an underlying primary digestive disorder (PDD) but also for IF secondary to primary non-digestive disorders (PNDD). PNDD diagnoses include other major organ failure, oncology and immunological disorders as well as organ transplants including bone marrow transplant. Please see Table 46.1 for aetiologies of intestinal failure (IF).

PN should be prescribed and administered in an appropriate clinical setting with multidisciplinary team (MDT) support [3]. Despite the success of well-managed PN treatment, if used incorrectly, there is a potential for harmful effects to outweigh the benefit to the child. Complications of PN can be life-threatening. In other words, PN is not just another drug to be prescribed. The support needed includes a comprehensive laboratory service, nutrition pharmacy, dietetics and microbiology support in addition to specialist medical and nursing skills [3].

When administering PN the aim of treatment is to stabilise the child at the same time as investigating, diagnosing and treating the underlying cause of the IF with the aim of regaining enteral autonomy. In some children with severe underlying disease unresponsive to treatment, long-term PN is continued at home. This chapter will also discuss when to consider intestinal transplant and the rare situation in which

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Table 46.1 Aetologies of Intestinal failure (IF)

Primary digestive disorder	Primary non-digestive disorder
Post-operative abdominal surgery	Prematurity
Short bowel syndrome (SBS). Aetiologies include: Necrotising enterocolitis (NEC) Gastroschisis Intestinal atresia Congenital short bowel/ antenatal volvulus Total colonic + small intestinal aganglionosis	Major organ failure For example, neurodegenerative disorders
Protracted diarrhoea with faltering growth Aetiologies include: Tufting enteropathy Tricho-hepato-enteric syndrome Microvillous inclusion disease TTC7a deficiency Other genetically inherited diarrhoea Autoimmune enteropathy Unknown aetiology	Chemotherapy Radiotherapy
Motility disorder: Paediatric intestinal pseudo-obstruction (PIPO) Intestinal dysmotility	Severe trauma Extensive burns
	Bone marrow transplant Systemic immunodeficiencies

it might be more appropriate to withhold treatment when a child has such profound ill health beyond the gastrointestinal tract that prolonging life with PN would increase suffering with no chance of recovery.

The chapter will focus on the use of PN in children and infants beyond the neonatal period. (Please see Chap. 53 for the use of PN in neonates and premature infants.)

Definitions of PN and EN

'Total' PN (TPN) is the term used for infusion of all the individual's nutritional requirements into the bloodstream. 'Partial' PN is an incomplete parenteral infusion of the patient's daily nutrient requirements. In most circumstances partial PN is given.

Intestinal failure (IF) has been divided into three groups: type I IF for acute self-limiting condition, type II when the patient is metabolically unstable and remains PN dependent for weeks or months and type III when a stable patient has a chronic underlying disease and requires long-term PN for months or years. Type III IF patients are usually discharged home on PN treatment [4].

The term EN will be used in this chapter to cover any food or artificial commercially available liquid feed that is taken by mouth or given into the intestine.

History and Development of PN

Attempts to feed directly into the bloodstream have been made ever since the circulatory system was first described by William Harvey in 1628. For example, in 1656 Sir Christopher Wren infused ale and opium into dogs using a bladder and sharpened quill. Survival was limited to a few days [5].

In order for PN to be safely used in the clinical setting, appropriate non-toxic protein, carbohydrate and lipid sources were required in a form that was stable in solution and could be infused directly into the bloodstream. In 1937 the first suitable protein source was developed when a casein hydrolysate was successfully infused in adults. In 1940 a crystalline L-amino acid solution was first used clinically in children. These solutions were eventually available for routine use in the 1960s by which time technical difficulties and high commercial costs were overcome.

Carbohydrate energy sources trialled included fructose, sorbitol, ethanol and glucose. Glucose was metabolically the most appropriate energy source but was associated with venous thrombosis. Then in 1968 Dudrick et al. found that by using a large central vein to infuse glucose, thrombophlebitis was avoided [6]. Despite this success it was not possible to provide sufficient calories with carbohydrate and amino acid alone in a small enough fluid volume for a child's needs. If PN were to be viable, a fat source was needed. An artificial 'chylomicron' composed of soybean oil and egg phosphatides was developed by Wretlind and Schuberth in 1963 [7]. As a result, PN was provided on a commercial basis from the late 1960s. The same soybean lipid is still in use today.

Constituents of PN

PN is a complex mixture of sterile nutrients in a suitable form to be safely infused directly into the bloodstream. It consists of carbohydrate lipid and protein with added electrolytes, vitamins and minerals. In 2018 an expert working group produced comprehensive guidelines on the use of PN in children on behalf of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), the European Society for Clinical Nutrition and Metabolism (ESPEN), the European Society for Paediatric Research (ESPR) and the Chinese Society of Parenteral and Enteral Nutrition (CSPEN) [8–15]. The guidelines provide comprehensive guidance on intravenous formulation and safe infusion.

The standard carbohydrate source used in PN is dextrose. The protein is an amino acid formulation based on egg protein and, in the neonate, on breast milk. Lipid is usually supplied as a separate infusion to which the fat-soluble vitamins, vitamins A, D, E and K, can be added. Water-soluble vita-

mins and trace elements are added to the amino acid and carbohydrate-containing bag.

The PN is formulated to provide the carbohydrate, amino acids and lipid according to the child's nutritional needs taking into account age-related requirements and nutritional status. It is formulated within the stability parameters for the constituents [8–15]. Whilst some constituents of the formulation are relatively stable, others can precipitate when exposed to high concentrations of certain nutrients. For example, sodium concentration can be increased without problem, whereas the concentrations of calcium and phosphate have to be carefully titrated [12]. If PN is made up in a two-bag system with separate lipid, calcium and phosphate are stable in a higher concentration than when in an all-in-one bag that includes the lipid.

For many years soybean was the sole lipid source. However, over the last 10–15 years, olive oil, fish oil and coconut oil have been used. (See Table 46.2 for some of the available mixed lipid emulsions.) Mixed lipid emulsions are the recommended treatment for children who need PN for more than a few days [9].

The advantage of soybean lipid is that it contains the essential fatty acids (EFA) alpha-linolenic acid (LNA) and linoleic acid (LA) in adequate concentration. However, it is relatively low in vitamin E and is a source of phytosterols that have been implicated in the aetiology of intestinal failure-associated liver disease (IFALD) [16]. (Please see IFALD below.) Lipid should represent about 30% of non-protein energy, and linoleic acid should be 1–2% of total energy. The maximum lipid utilisation rate is about 3.3–3.6 g/kg/day. In order to prevent EFA deficiency in term infants and in children, a lipid emulsion dosage providing a minimum LA intake of 0.1 g/kg/day can be given, which also provides an adequate intake of linolenic acid with all ILEs currently registered for paediatric use [9] (see Table 46.2).

Trace minerals and vitamins are available in mixed formulations or can be added individually to PN [13]. They are usually incorporated in bespoke PN during the manufacturing process but need to be added to standard bags prior to infusion. Iron is not routinely included in view of the risk of iron-associated liver cirrhosis. However, iron can be added in children on prolonged treatment who are unable to tolerate an enteral iron supplement [13]. Certain additional substances can be directly added to the formulation such as the non-essential amino acid glutamine and certain medication, such as the H-2 receptor antagonist, ranitidine [15].

Table 46.2 Examples of parenteral nutrition lipid solutions[a]

Clinoleic 20% (Baxter): 20% soybean oil, 80% olive oil
Intralipid: 20% (Baxter) pure soybean oil
Lipidem (B. Braun): fish oil, olive oil, soybean oil
Lipofundin (B. Braun): medium-chain (MCT) and long-chain triglycerides (LCT) MCT/LCT®: 20% 1:1 weight, soybean and coconut oil
Omegaven 20% (Fresenius Kabi): pure fish oil[b]
SMOF lipid® 20% (Fresenius Kabi): 30% soybean, 30% MCT, 25% olive, 15% fish oil

[a]These are examples and not a comprehensive list
[b]Omegaven is not licensed for sole lipid use and should be given alongside another lipid infusion other than in exceptional short-term circumstances

PN should be formulated in a licensed compounding unit. The unit should have access to their own stability data based on available guidelines from national and international guidelines [3]. An automated system in a specialised sterile unit is usually used to mix the PN ingredients according to international pharmaceutical standards. The final formulation is supplied in a sealed sterile bag. The nutrients for a 24- or 48-h period are contained within one or two bags. If in two separate bags, one will contain the amino acids, dextrose and electrolytes, either with or without added trace elements and water-soluble vitamins; and the other lipid- and fat-soluble vitamins.

Stability of the PN formulation can be a major problem in children when compared to adults since the child has a relatively high calcium and phosphate requirement: two salts that readily precipitate [12].

Types of PN Formulations and Pharmacy Arrangements

1. A standard age and body weight-appropriate bag. Standard pre-prepared commercially available bags are the treatment of choice for all patients other than those with complex intravenous needs [17]. The lipid emulsion and solution containing amino acids and dextrose are usually in two separately sealed compartments of the same bag. The user can break the seal between the two (so that the bag becomes one) if the patient requires both to be infused or just infuse the amino acid and dextrose and discard the lipid section. If the patient requires extra electrolytes, a certain amount of, for example, sodium can be added to certain bags immediately prior to infusion, preferably in a sterile compounding unit in the hospital pharmacy. Vitamins and trace elements usually need to be added to the bags or given enterally. Most patients even with profound IF can absorb sufficient vitamins and minerals unless totally nil by mouth or high gastric losses. The manufacturers' instructions need to be followed with regard to additions. Standard bags can have a shelf life for several days, weeks or months, and some can be stored at room temperature. There are an increasing variety of standard paediatric bags available.

2. Individually formulated/bespoke PN bags are the most appropriate treatment for unstable hospitalised children with unpredictable variation in daily fluid and electrolyte needs. They are usually prescribed and manufactured on a daily or 48-h basis according to the patient's requirements. The 'shelf life' of these bags is usually approximately 48–96 h. This bespoke PN is normally made up as two separate bags: (1) amino acid and dextrose with electrolytes, trace elements and water-soluble vitamins and (2) lipid with fat-soluble vitamins. The pharmacy manufacturing of PN is usually within the hospital.
3. Certain PN manufacturing pharmacies have sufficient expertise and stability data to provide individually prescribed PN with prolonged stability for up to about 4 weeks. All-in-one bags with the lipid, vitamins and minerals in a single compartment together with the amino acids and dextrose are manufactured to the patient's needs. There will be a gradual breakdown of certain vitamins, for example, photo-degradation of vitamin A during storage. However, with regular (usually 3 monthly) monitoring of blood levels, vitamin levels can be maintained in the normal range. These pharmacies usually supply PN to patients with chronic IF on long-term treatment who are at home [18].

When to Start PN

It is essential that all attempts are made to feed a child enterally unless contraindicated, e.g. intestinal obstruction or ileus post-gastrointestinal surgery, prior to commencing PN. Once the need for PN has been established, it usually needs to be commenced promptly. Nutritional support should be begun within 1–5 days if a child is unable to tolerate EN and is not expected to recover intestinal function within the next few days.

Exceptions to early PN introduction are the acutely unwell child with multi-organ failure and children in intensive care during the first week of admission. A randomised controlled multicentre trial of 1440 children admitted to intensive care recorded a significantly better outcome for children if PN was delayed until the second week [19]. However, the children were given micronutrients, i.e. vitamin, mineral and trace element supplements during the first week.

How to Start PN

When starting PN the child's age, weight and length/height, nutritional state, intestinal losses, other infusions, any enteral feed and fluid tolerance all need to be taken into account. It is usually the best practice to stabilise the patient and start PN during normal working hours (other than in the premature neonate). (See Table 46.3 for the steps that need to be taken when commencing PN.)

Table 46.3 How to start parenteral nutrition (PN)

1. Obtain secure central venous access
2. Assess maintenance fluid and electrolyte requirements from patient's length/height and weight[a] (estimated height/measurement in the last 3 months may be used if the child is critically ill and can't be measured)
3. If uncertain of accurate weight, e.g. fluid overload and not possible to accurately measure the child, check mid-upper arm circumference (MUAC)
4. Check whether there are cardiovascular, respiratory and/or renal abnormalities that might limit fluid tolerance and hence volume of the PN infusion
5. Check blood electrolytes, Ca, PO4, Mg and urine Na, and adjust electrolyte levels in PN accordingly
6. Add extra fluid and electrolyte requirements for excessive intestinal losses, or if the losses are highly variable, give as a separate infusion
7. Subtract volume of other treatments infused, such as Na, fluid and dextrose infused with, e.g. inotropes and antibiotics[b] If the child has residual absorptive capacity and remains on partial EN, the volume and nutrient content should be taken into account as well although only partial absorption is usually assumed
7. Prescribe PN using the fluid volume available adding nutrients according to the pharmaceutical guidelines for concentrations of constituents
8. Day 1: start with 60% requirements, and increase to full PN constituents prescribed over 4–6 days in order to limit risk of refeeding syndrome
9. Obtain appropriate equipment for infusing PN: a giving set to connect the central venous catheter to the bag of PN + an infusion pump
10. Place a filter in the giving set: Lipid emulsions and all-in-one mixes membrane pore size of 1.2–1.5μm Aqueous solutions 0.22μm
11. Monitor weight and blood electrolyte levels on a daily (or twice daily) basis for the first 5–7 days

[a]Parenteral fluid and electrolyte requirements are prescribed using the child's weight. However, the actual weight in a sick child may be overestimated if the child is fluid overloaded or under-estimated if there has been weight loss. By plotting the height/length on the centile chart, the appropriate weight for the child can be estimated, i.e. the same centile for age as the height. The patient's PN can then be prescribed using that weight as the prescribing/working weight. If the weight is very different to the child's current weight, e.g. the severely under-nourished/wasted child, a weight between the actual and expected weight can be used in the first instance and gradually increased towards the expected weight as the child's nutritional state improves

[b]Children in intensive care often have restricted total fluid intake and require other intravenous infusions in addition to PN. It may not be possible to give the required nutrients in the volume available for the PN infusion. Once the child improves clinically, fluid restrictions and infusion volumes are eased and the PN can be increased

Venous Access for PN Infusion

In order to safely administer PN with a sufficiently high concentration of nutrients for a child to thrive, the PN needs to be infused through a central venous catheter (CVC). The tip of the catheter should be sited low in the superior vena cava above the pericardial sac (or above the first lumbar vertebra, i.e. above the renal veins if inserted via a femoral vein) [20]. The appearance on the chest x-ray should be with the tip 1 cm above the carina in the older child and 0.5 cm in smaller children and infants [20]. The catheter is usually placed via the internal jugular, subclavian or femoral vessels or via a peripheral vessel. The femoral veins are less suitable than the superior vessels, since the exit site is in the nappy area and susceptible to faecal contamination (unless the catheter is tunnelled under the skin). In general, the CVC should be tunnelled under the skin to reduce the risk of skin microbiota infecting the bloodstream [20]. Ideally a single-lumen catheter should be used in order to limit the risk of infection. In certain children who depend on multiple intravenous infusions, a double-lumen catheter may be required. Catheters made from silicone or polyurethane should be used in preference to those from other materials since more flexible. A cuffed catheter is preferred to hold the catheter in situ with subcutaneous positioning of the cuff. However, an uncuffed peripherally inserted central catheter (PICC) with the tip centrally placed can also be used, particularly if it is likely to only be needed for several days or a few weeks rather than for several months. The major advantage of an uncuffed catheter is that it can be removed without the need for general anaesthesia. Although the use of a PICC is only recommended for a few weeks, it is possible for a PICC to function successfully for longer, even in children at home on PN.

A subcutaneous PORT is not usually appropriate for PN infusion since access is via a transdermal needle that remains 'in situ' when it is used [20]. If the needle becomes dislodged, the PN may be infused into the tissues with significant tissue damage that can require plastic surgery. The needle should be checked regularly during infusion which is not feasible in a child cared for by parents at home.

It is the best practice to insert the central venous catheter (CVC) under radiological control in order to minimise damage to the blood vessel during insertion. Limiting vascular damage is particularly important if the patient might require long-term PN. Loss of patency of major blood vessels is one of the indications for referral for intestinal transplant.

In some children on long-term home treatment, the CVC can remain 'in situ' for 8–10 years or longer. The CVC may eventually need to be changed routinely when the tip becomes displaced from the superior vena cava due to the child's growth. There is a risk of the CVC becoming embedded in the wall of the blood vessel with the need for removal by a vascular surgeon. In view of this risk, elective CVC change may need to be considered before 10 years in situ. Ideally the CVC should be dedicated to the infusion of PN in order to preserve patency and minimise the risk of CRBSI. In children who have had frequent cannulation of blood vessels and/or excessive anxiety associated with venepuncture, it may be extremely difficult to obtain blood samples from a peripheral vein. The distress and/or time attempting peripheral venous access may outweigh the potential complications of using the CVC for blood sampling and/or other infusions.

PN Infusion and Cycling

1. The PN should be infused via a pump. In hospital static pumps are used, whereas the child at home benefits from a portable pump with a backpack for the PN bag itself [18]. The pump should be positioned within 30 cm of the heart. The infusion should pass through a 1.2-μm filter if a lipid contains infusion and a finer 0.22-μm filter if lipid-free [3]. The infusion is usually over 24 h when PN is commenced.
2. As soon as the child is sufficiently stable, a reduction in infusion hours should be trialled. Infants weighing under about 4.5 kg are unlikely to tolerate even 1 h off PN unless they are absorbing at least 20–30% of nutrients enterally. To start cycling PN, the infusion rate should be 'wound down' during the final hour of infusion in order to limit the risk of hypoglycaemia. The initial wind down would be to reduce the PN rate to 50% for 30 min and then 25% of the original rate for the final 30 min after 22 h of infusion and to have 1 h free from the infusion. The blood sugar level should be checked during the hour off PN. If there is no evidence of hypoglycaemia, the PN can then be reduced by a further hour every few days aiming for 12 h of minimum infusion.

Monitoring on PN Treatment

The key to successful treatment with PN is careful monitoring. Monitoring should include clinical observations, urine and blood biochemistry and haematology and anthropometry. (Please see Table 46.4 for details.) Patients should also have daily medical and dietetic review.

Role of the Nutrition Support Team

It is important to bring all professionals contributing to the management of the PN together to work as the nutrition support team (NST) [3]. The team usually comprises specialist

Table 46.4 Monitoring the hospitalised patient on PN treatment

Clinical observations: Temperature, heart rate, respiratory rate, blood pressure, 6 hourly minimum
Ward urine analysis: Glucose and ketones until stable[a] Re-start checking urine ketones if PN glucose concentration increased
Weight[b] (ideally on the same scales and at the same time of the day): Daily for 5 days (twice daily if excessive fluid losses/difficult fluid balance) Twice weekly once stable and then weekly
Blood sodium, potassium, urea, creatinine, calcium, magnesium phosphate, liver function, full blood count and triglycerides: Minimum daily for 5 days Twice weekly once stable and then weekly
Urine sodium[c]: Twice weekly and then weekly once stable Re-check 24–48 h after increasing sodium in PN if level was previously low
At start of cycling PN: Check blood glucose regularly, e.g. initially at 30 min intervals after stopping the infusion
Long-term in-patient treatment > 27 days repeat 4–6 weekly: Zinc, copper, selenium + CRP Vitamins A and E, clotting studies, vitamin D Ferritin, iron, TIBC Anthropometry: length/height, weight, mid-upper arm circumference Head circumference in children <2 years of age
1. *PN treatment > 3 months consider*: Thyroxine, thyroid-stimulating hormone (TSH) Carnitine Vitamin B 12

[a]The maximum infusion rate of glucose through a central vein should be 1.0–1.5 g/kg/h; if greater than this, glycosuria is highly likely
[b]In certain conditions when fluid balance is altered, weight may not be a useful measure. For example, children in renal failure or with low blood albumin may retain fluid within the body tissues and those with intestinal pseudo-obstruction may 'pool' fluid within dilated intestinal loops
[c]inaccurate if on diuretic treatment

physicians, pharmacists, dietitians and nurses with access to a microbiologist, laboratory support and additional professionals as required. There could be a need for a paediatric surgeon, psychologist, social worker and/or speech therapist.

The overall aim of treatment with PN is to support appropriate weight gain and growth whilst minimising complications (related to underlying disease as well as to PN) and aiming for intestinal autonomy.

Roles of the team include reviewing individual patients on PN treatment, education, setting local guidelines and following international guidelines.

All children receiving PN treatment should be reviewed on a regular basis and at least weekly by the NST [21]. The team may either prescribe or advise the medical team caring for the patient. The NST will participate in ensuring the child gains and maintains a good nutritional state, participate in managing the complications of PN and encourage and support weaning from PN if achievable. They will usually support the patient through four phases of treatment:

1. Stabilise the patient.
2. Aim for appropriate weight gain—usually 'catch up' weight gain required.
3. Maintain weight centile appropriate for patient's length/height.
4. Withdraw/wean PN and give increasing volume of oral food or liquid enteral nutrition.

The most important complications are described below:

Complications of PN

The most frequent complications associated with PN are metabolic, inappropriate weight gain, infectious, catheter-related, liver disease and thromboembolic disorders. Each of these problems will be discussed below.

Metabolic Disorders

Common metabolic problems are refeeding syndrome, electrolyte abnormalities and inappropriate weight gain. Initial PN complications are most commonly related to fluid and electrolyte imbalances [22].

Refeeding syndrome develops when full nutrition is commenced too rapidly after a period of extremely poor nutritional intake. As the body changes from catabolism to anabolism potassium, phosphate and magnesium are taken up into the cells with a rapid reduction in blood levels. If severe, refeeding syndrome can be life-threatening. The maximum possible phosphate will be needed in the PN. An oral or peripheral blood infusion of phosphate and/or potassium may be required alongside the PN if the amount required exceeds the concentration that is stable within the pharmacy guidelines for PN formulation.

Inappropriate Weight Gain

Inadequate weight gain is a frequent problem with PN. Four possible reasons are (1) fluid restriction, (2) infusing a lower volume of PN than that prescribed, (3) under-prescribing and (4) negative sodium balance or low total body sodium.

It may not be possible to infuse sufficient volume of PN to supply adequate calories and protein in the critically ill child with other major organ failure even when the PN formulation is at the maximum concentration when the child is fluid restricted. The child may only tolerate limited fluid volume, and a proportion of the fluid that they can have may be taken up with other infusions such as inotropes and antibiotics. It may be necessary to infuse the medications in the minimum

possible fluid volume and to ensure that whenever possible medications are prepared in 10% dextrose to maximize calorie intake.

Secondly, the volume of PN actually infused is often less than the volume prescribed. For example, the PN infusion may be stopped to infuse other treatments through the same CVC lumen. The length of time taken for those infusions needs to be taken into account when calculating the rate of the PN infusion to ensure the total PN volume required is prescribed in the hours available.

The PN prescription is usually based on body weight. It is important that the prescriber uses the patient's expected weight for length/height rather than the actual weight. If the weight centile is significantly lower or higher than the length/height centile the appropriate weight for height should be estimated, i.e. check the child's height/length centile on the growth chart and use the weight for the equivalent centile to the height/length for calculating PN requirements (see Table 46.4).

Low total body sodium is another frequent longer-term problem, particularly when intestinal stoma sodium losses have not been adequately replaced. Measurement of urine sodium is one of the most helpful investigations in the absence of diuretic treatment or renal failure. Patients with a low urinary sodium level, < 10–20 mmol/l, may have a low total body sodium and are unlikely to gain weight appropriately [23]. Extra sodium may need to be added to the PN infusion. If a patient is weaning from PN, the sodium supplement is best given enterally.

It is also important to be aware of over-feeding. Excess nutrients and in particular carbohydrate and lipid have been associated with liver disease [24] and carbohydrate with increased carbon dioxide production and worsening respiratory function [8].

Infection

Infection associated with PN may be a bloodstream infection related to the catheter, a catheter-related bloodstream infection (CRBSI), infection of the skin and/or sub-cutaneous tissues around the catheter exit site or rarely in the PN formulation itself.

CRBSI is one of the most common potentially life-threatening PN complications. In order to minimise the risk of the child becoming seriously unwell, the condition needs to be recognised as soon as it starts to develop and treatment commenced without delay. Symptoms include a fever of 38 °C or higher or other signs suggestive of septicaemia in that particular child (some children do not have a high temperature even with CRBSI). Blood for culture should be taken via the CVC and if possible peripherally as well. At least two broad-spectrum antibiotics should be commenced to treat potential gram-positive and gram-negative bacterial infection. The antibiotics need to be administered via the CVC. If there is more than one lumen, then treatment should be rotated between the lumens in order to eradicate the infection. Once cultures are available, the antibiotic regime should be tailored according to the bacteria detected. For example, if the organism is only cultured from one lumen, then treatment should only be given via that lumen. In children (other than neonates or those with a significant immunodeficiency), the antibiotics can be stopped if cultures are negative after 48 h. PN should be continued whilst treating the infection unless the child develops severe cardiovascular compromise when the infusion is commenced. A 'Y' connector needs to be attached to the 'giving set'/infusion tubing for antibiotic administration.

In order to limit the risk of CRBSI, the CVC connections and disconnections should be kept to a minimum and only done by trained professionals. The CVC hub should be cleaned with 2% chlorhexidine when connecting and disconnecting the CVC [25]. The skills of professionals who access CVCs should be regularly reviewed and updated as necessary.

There is also the risk of infection of the skin at the catheter insertion site that can track along the subcutaneous portion of the catheter. If insertion site infection fails to respond to antibiotic treatment, the catheter may need to be removed. Rarely the PN formulation itself can be contaminated during the manufacturing process despite all the sterile pharmaceutical precautions taken. Treatment strategy would be the same as for CRBSI. The child is of course also at risk of the usual acute childhood infections.

CVC Occlusion and Venous Thrombosis

Catheter-related complications include catheter blockage, breakage and displacement. Central venous catheter (CVC) occlusion may occur with deposition of lipid and/or calcium and phosphate precipitates within the lumen. Obstruction can also be positional if the tip of the CVC rests against the wall of a blood vessel. Occlusion may be cleared with alteplase, urokinase or alcohol line lock [26]. Limiting lipid infusions to two or three times a week helps prevent CVC occlusion related to fat deposition.

Thrombosis is a rare but potentially serious complication. It may involve the catheter itself or blood vessels. It can present with difficulty when flushing the catheter. A child with superior vena caval obstruction may have facial puffiness. Ultrasonography is used to diagnose smaller vessel thrombosis whilst venography is required for diagnosis of major vessel blockage. If the catheter alone is thrombosed, a streptokinase or urokinase flush may be used. Alteplase and interventional radiological techniques may be required to

unblock major venous thrombosis. If treatment is unsuccessful and there is loss of access to four or more major veins, intestinal transplant assessment is needed [27]. Pulmonary embolus (PE) is a rare complication that should be considered in the child on PN treatment who is acutely unwell with no clear aetiology or classical PE symptoms[28].

Liver Disease

A common and potentially life-threatening complication is IF-associated liver disease (IFALD). Risk factors of IFALD include prematurity, lack of enteral intake, recurrent CRBSI, other infections, length of time on PN treatment, overfeeding, intestinal bacterial overgrowth, certain drug treatment and certain components of PN [29]. Constituents of PN solutions implicated include a high carbohydrate load and the soya component of lipid. Soybean contains phytosterols that have been associated with liver disease by inhibiting bile acid secretion [16]. Management strategies for limiting IFALD are shown in Table 46.5. Reducing the hours of PN infusion and limiting lipid to alternate days appear to improve liver function [26]. The use of newer mixed lipid emulsions (see Table 46.2) including olive oil, MCT and/or fish oil as well as soybean has also been associated with less IFALD [30]. Safety data using mixed lipid types have been published for premature infants and children [31]. When affected patients have changed to pure Ω-3 fish oil-based lipid (Omegaven 10%, Fresenius Kabi) infusions, severe cholestasis has improved or even resolved [32].

Table 46.5 Management to minimize intestinal failure-associated liver disease (IFALD) [9, 24, 29, 30]

1. Introduce enteral feed at the earliest opportunity Enteral feed should be continued for intestinal integrity in addition to the protective effect on the liver even if only small volumes are tolerated
2. Cycle PN at the earliest opportunity (see infusion section above)
3. Administration of a mixed lipid emulsion with a good omega 3/omega 6 ratio including fish oil: If totally dependent on PN, ensure minimum LCT lipid for adequate essential fatty acids If PN needed for >14 days, use a mixed lipid emulsion In cholestasis avoid pure soya lipid even if PN is only needed for a few days Limit the lipid infused or stop completely if patient is tolerating some enteral nutrition or unlikely to need the PN for > a further 14 days Reduce the number of lipid infusions/week to, e.g. alternate days or just 2–3 times/week at the earliest opportunity
4. Treat any underlying infection promptly: Even with appropriate treatment, there may be a temporary increase in liver enzymes with CRBSI or other intercurrent infections
5. Consider ursodeoxycholic acid at 10 mg/kg TDS
6. If there is evidence of intestinal bacterial overgrowth, investigate and treat

Enteral feeding even if less than 10% of the total energy intake is an important factor in preventing/reversing cholestasis [29]. Ursodeoxycholic acid may be beneficial although it is not always well tolerated. Small intestinal stasis can be associated with bacterial overgrowth and if present should be treated [30]. Copper and manganese are usually excreted in the bile and can become hepatotoxic in cholestatic patients. Please see Table 46.5 for management strategies for limiting IFALD.

If IFALD is persistent and worsening, early referral for assessment by an intestinal transplant centre is recommended [2].

Enteral Nutrition and Weaning from PN

Enteral autonomy is usually achieved in four stages:

1. On starting PN aim to stabilise the patient.
2. Aim for appropriate weight gain on PN—usually 'catch up' weight gain required.
3. Maintain weight centile appropriate for patient's length/height.
4. Withdraw/wean PN and give increasing volume of oral food or liquid enteral nutrition.

The aim is to give a mixed diet by mouth with the range of foods and amount eaten tailored to the patient's enteral tolerance with the ultimate aim of weaning off PN and onto diet alone.

Enteral nutrition is given alongside PN in two contexts:

1. Early introduction of oral food or EN as trophic feeds should be given alongside PN treatment if at all possible [3]. The major benefits of minimal/trophic enteral nutrition include:
   - Prevention of intestinal mucosal atrophy
   - Maintenance of the enterohepatic circulation (see Table 46.5)
   - If given by mouth, retention of feeding skills

However enteral nutrition will need to be limited if there are significant adverse consequences such as excessive fluid losses, vomiting or abdominal pain associated with its introduction.

2. Secondly, once a child is tolerating EN well, the possibility of increasing EN and weaning from PN is considered. At this stage the child should be in an improved nutritional state. The aim should be to reduce the PN and give the maximum EN tolerated.

It is important to be purposeful when weaning. There are a variety of weaning strategies that can be used. Please see Table 46.6.

Table 46.6 Strategies for weaning PN [3, 33]

The speed of weaning from PN varies widely: If severe IF, feed volumes may need to be increased slowly, according to the digestive tolerance, e.g. an increase of 1 ml/h every 24 h Some other children can abruptly return to full nutrition over a few days Children with chronic IF on PN at home will often need gradual introduction of enteral nutrients over weeks or even months, e.g. severe SBS
The weaning plan should be made with the MDT
Ensure parents are aware of the risk of continuing PN versus the benefit of weaning Involve the parents/carers in drawing up the weaning strategy
The smaller infant will almost certainly need an artificial feeding device: the volume of feed needed to wean is usually so great that the child will not willingly ingest sufficient orally
Maximise absorptive ability with a liquid enteral feed given continuously via tube feeding with a volumetric infusion pump or 2 hourly boluses in a small infant
Allowing the older infant to breast/bottle-feed and have solid food and the child to eat and drink rather than giving a liquid enteral feed is usually more successful
If oral feeding is contraindicated, a liquid enteral feed should be given
Make one change at a time
In the first instance, avoidance of common dietary antigens can be beneficial, e.g. avoidance of cow's milk, egg, wheat and soya. Slow onset/non-IgE-mediated food allergy is common in infants with an enteropathy and in SBS secondary to NEC or volvulus probably subsequent to ischaemic damage
Prioritise the weaning rather than risking setbacks by introducing a new food that precipitates symptoms that interfere with the weaning process. Once the PN has stopped, the diet can be expanded again
Offer food little and often. A child with ultra-SBS may not tolerate a full meal
It is usually possible to stop a night of PN if clinically stable on about 50% requirements enterally
If the child is lethargic the day after a night without PN, the most common problem is sodium deficiency [33]: 1. Check urine sodium the a.m. after the night off PN 2. Give oral/enteral NaCl (1 mmol/ml) usually starting with 1–2 mmol/kg 3. If larger amounts Na needed 30% NaCl (5 mmol/ml) can be used
If the child has large intestinal fluid losses and poor fluid intake, oral rehydration solution may be more appropriate than a concentrated sodium solution
If high output, proximal stoma/jejunostomy trial double concentration Dioralyte/oral rehydration solution [34]
Never be complacent about the child's safety on PN even if they have not had any significant complications such as CRBSI. There is always a risk whilst the CVC is in place

The child's weight needs to be checked regularly, for example, twice weekly in the hospitalised child and once a month in the child weaning slowly from PN at home. It is important to be aware whether the child can afford to lose weight during the weaning process. Many children on PN are kept on a centile that is above their height/length centile. It is acceptable for their weight to fall to the equivalent centile to their height when weaning. As long as the child was initially in a good nutritional state, it will not harm them to lose weight at the time of weaning.

When weaning the older infant or child, it is usually best to reduce the volume of the PN first, and the child will then hopefully start to eat and drink more (or if on a liquid enteral feed, tolerate an increase in volume). The PN is often best reduced gradually, e.g. by 10% increments initially. Once the child is managing about 50% of requirements enterally, a whole night without PN can be considered in a child who can eat and drink. If the child is dependent on a liquid feed, the reduction might be continued more gradually.

Failure to Wean from PN as Expected

When it is not possible to wean a child from PN who appears clinically ready to do so, the following investigations should be considered:

1. Re-check urine Na since this is a good indicator of total body sodium (unless on diuretic treatment) + urine specific gravity. Check the first sample after disconnecting PN in the morning. If low, start an oral/enteral Na supplement. A child with a low total body sodium is unlikely to thrive [23].
2. Dietary assessment by a dietitian with IF experience.
3. Faecal reducing substances for sugar malabsorption if watery diarrhoea/high stoma losses.
4. Stool elastase for pancreatic malabsorption.
5. Intestinal endoscopy with mucosal biopsies for histological (and in certain cases electron microscopy) examination.
6. Urine organic acids should be checked for bacterial metabolites and blood for D-lactate and consider H2 breath test since intestinal bacterial overgrowth may present with worsening gastrointestinal symptoms and lethargy.
7. Consider a radiological contrast study to exclude strictures and other abnormalities particularly in the child who has had previous intestinal surgery.
8. Consider the possibility of fabricated or induced illness. If suspected, one-to-one 24 h nursing care may be needed as well as limiting the mother's involvement in the child's care.

Preparation for Home PN: Care in Hospital

Whilst most patients wean from PN regaining intestinal function within days or weeks of starting PN treatment, there are a small number who become dependent on PN for longer. These children should be considered for discharge home on treatment with PN if after extensive investigation and treatment by a specialist multidisciplinary paediatric gastroenterology service, it is not possible to significantly improve the

underlying disease, and the IF is expected to persist for at least two further months. Every attempt should have been made to wean the child from PN using the most appropriate type of feed given via the most suitable feeding device. The patient should be assessed by the IF rehabilitation service for home PN if not already under their care.

Many services will discharge an infant home from about 4 months of age at which stage the infant should tolerate several hours off the PN infusion. However, some services discharge even younger infants once they are stable, even when still on continuous PN [35]. The use of portable pumps has improved mobility for children when connected to PN. Most infants are able to tolerate a 10-h period without PN from about 4.5 kg (if weight is in the normal centile range for age), depending on their enteral tolerance and nutritional state.

The aetiology of chronic IF requiring long-term/home PN is most commonly short bowel syndrome (SBS). Infants with ultra-SBS, i.e. with < 10 cm in length of post-duodenal small intestinal remnant after surgical resection will almost certainly require long-term/home PN. Home PN is also needed for children with other PDD and for some PNDD such as life-limiting severe immunodeficiency, chronic graft versus host disease post bone marrow transplant and IF secondary to severe neurological impairment, please see Table 46.1.

Treatment at home with care by parent(s)/carer(s) who have been formally trained to administer PN and instructed what to do if complications arise gives the child the best chance of long-term survival with minimal complications [18]. It has been clear for some years that the advantages of homecare include improved quality of life, reduced incidence of CRBSI [36] and IFALD, improved psychosocial circumstances and reduced cost of treatment [18]. The aim of PN at home should be to incorporate the child's care into the family's lifestyle and not to have a 'hospital at home'. The child can attend school and participate in other childhood activities including swimming. It is also possible to enjoy family holidays again. Most parents will return to their previous employment.

However, the home environment needs to be a safe place for PN administration. Ideally there should be running water easily accessible from the child's bedroom, reliable electricity supply and sufficient space to connect/disconnect PN. At least one parent/caregiver (and if at all possible two) should to be formally trained. In certain circumstances a nurse may visit the home to connect and disconnect PN, twice daily. Appropriate social support should be organised (including financial support in many countries) and arrangements for appropriate home equipment including a portable pump. In many countries a homecare company supplies the PN and related equipment. Ideally PN should be supplied in a single bag that includes the lipid with the vamin-dextrose to simplify care with only one infusion pump needed. Shared care should be set up with the most local hospital to the child's home and the specialist centre (if the specialist centre is not close to the families' home) in order to ensure treatment can be started promptly for emergencies such as suspected CRBSI [18].

The current UK guidelines recommend that a viable home PN service should manage at least 10–20 patients with an MDT [37]. The composition is similar to the hospital MDT described above. Table 46.7 gives details of the steps needed to arrange transition from hospital to homecare. Good communication is essential for successful management of PN at

Table 46.7 Preparation for discharge home [18]

1. The patient's hospital team and the IF rehabilitation team need to meet to ensure treatment is rationalised to the simplest possible management plan
2. The IF rehabilitation doctor and nurse should meet with both parents (unless the father is unknown), even if they live apart It is important to establish the need for both parents to be involved in the child's care It also gives the home PN team the opportunity to explain the child's condition and future care plan and prognosis to both parents It is best to train both parents even if one of them will lead on care The other parent should aim to do at least one connection/disconnection each week to maintain skills
3. If there is only one parent available, a second relative may be prepared to be trained in order to support the single parent. If parents are physically unable to perform the procedure, a community-based nurse may do so
4. Aim to reduce PN infusion time to a minimum of 12–14 h overnight if possible
5. Ensure good venous access with single-lumen tunnelled CVC. A PICC line can be used at home if functioning well. Doble lumen CVC may be used if other intravenous infusions also required.
6. Arrange a home visit to check facilities for managing PN: Running water easily accessible from the child's bedroom Reliable electricity supply Sufficient space to connect/disconnect PN Space for a dedicated fridge to store PN bags (may be outside the house)
7. Arrange funding and source a home PN supplier: Arrangements vary from country to country. Currently, in the UK, there is a national framework for home PN with central government funding In many countries (including the UK), a commercial homecare company manufactures and delivers the PN, and ancillary equipment including the fridge to the patient's home The PN formulation should have a minimum of 7 days of stability and preferably 21–28 days Deliveries limited to twice monthly if sufficient stability available
8. A specialist nurse should undertake the training programme with the parent(s) or carer(s) to cover the process of connecting and disconnecting PN and a plan of what to do when complications arise: The parent/carers need to be available for a 30-h training programme over a 10-day period Training may be completed sooner or in some cases take longer In the unlikely event that a parent fails to train, they may be offered a second training period after discharge home and watching their partner or a nurse connecting/disconnecting for a few weeks A one-person aseptic non-touch technique (ANTT) for connecting and disconnecting the catheter enables just one parent/carer to perform the connections and disconnections in the simplest manner possible
9. Shared care needs to be formally set up with the most local hospital for acute medical emergencies (e.g. suspected CRBSI or CVC displacement): Parents would usually continue to connect and disconnect the PN and connect other infusions/antibiotics to limit risk when in hospital

Table 46.7 (continued)

10. A discharge date should be set and a pre-discharge meeting arranged via video, telephone or face to face with parents present Attendees include the IF rehabilitation team specialist doctor and nurse, a community nurse, a local hospital consultant, parents and other professionals as needed. Please see Table 46.8 for meeting agenda. Minutes should be sent to all participants including parents
11. The child should be discharged home as soon as the parent/carer training has been completed (unless intercurrent medical problems arise) and if feasible after a 48-h period of self-care in hospital

Table 46.8 Points for discussion in the discharge meeting [33]

1. Patient's clinical history
2. Patient's current medical needs
3. Date of local hospital and IF rehabilitation team first out-patient clinics
4. Background information about the home PN service (non-specialist professionals may have never dealt with home PN before)
5. Create an action plan for parents to follow if/when problems arise: Fever or other symptoms suggestive of CRBSI Blocked, broken or displaced CVC
To discuss any psychosocial concerns and plan for support if needed
To ensure the different professionals and the family know how to contact the specialist centre
To arrange a date for a follow-up meeting 3–6 months after discharge should it be needed

home. A pre-discharge planning meeting is instrumental in setting up good homecare. Please see Table 46.8 for the meeting agenda.

Management at Home

Once established on PN at home, the focus is to ensure the child thrives with appropriate weight gain. At the same time, there are two main aims of treatment: firstly, to wean off PN by introducing enteral nutrition (EN) at the earliest opportunity and, secondly, to ensure that the child has the best possible quality of life whilst on PN.

Regular monitoring is essential to ensure treatment is given in the most efficient manner possible, to prevent complications (and if any do occur to detect them at the earliest opportunity) and to document changes in the patient's overall clinical condition [18].

Monitoring needs to include the patient's weight gain and growth, any metabolic abnormalities, infection, and liver and thromboembolic disorders [18]. Routine blood tests can be done as infrequently as once every 3 months in the stable child on PN at home.

Complications of Long-Term/Home PN and Their Management

Children at home are susceptible to the same complications as children on short-term hospital PN but are less likely to have certain metabolic problems and have some additional longer-term issues. The most common longer-term PN complications are CRBSI, displacement, breakage or blockage of the CVC, metabolic bone disease and IFALD [38].

Parents/carers should have the details of the nearest acute paediatric centre to take the child to have a blood sample taken for culture and two broad-spectrum antibiotics commenced if CRBSI is suspected. Treatment should be rationalised once a positive blood culture is available and stopped if the culture is negative after 48 h (see CRBSI above) after discussion with the specialist IF rehabilitation service.

In patients who have > one CRBSI at home, prophylactic anti-bacterial line locks such as taurolidine [39], ethanol or EDTA [40] should be considered.

The local acute paediatric service would usually manage a blocked CVC with an alteplase or urokinase infusion [2]. They should also manage treatment of infection of the CVC insertion site or subcutaneous infection along a tunnelled CVC. The local service would also be expected to stabilise a child with a displaced or fractured CVC prior to transfer to the specialist centre for CVC repair or replacement. Treatment should be discussed with the specialist service.

Other complications of long-term PN treatment are poor bone mineralization [41], abnormal body composition [42], gallstones [43] and rarely thrombotic complications such as pulmonary emboli [28].

Monitoring for potential bone disease includes blood ALP, Ca, P04 levels every 3 months if stable, vitamin D and parathyroid hormone every 6 months and bone age annually. From the age of 5 years (when there is a normal range to compare), bone density can be checked annually [18].

Management of IFALD has already been described under 'optimising PN management in hospital'. Additional investigations at home include an annual ultrasound scan to investigate for gallstones as well as liver parenchymal disease. Early referral to a hepatology transplant service is necessary if liver disease develops.

Specific Features of PN at Home

In the same way as in hospital, the hours that the PN is infused should be limited to the minimum possible, usually 12 h overnight. The child and family can participate in usual daytime activities. It can be difficult for the family to pursue a reasonably normal lifestyle if a child is connected for more than 14–16 h, although portable pumps facilitate improved mobility.

Every effort should be made to supply all PN requirements in a single bag for each infusion night in order to have one giving set and infusion pump. Most children will have two separate formulations to be used on different nights of the week since the lipid-containing infusion is usually limited to two or three nights/week, with the dextrose and amino acid preparation without lipid given on intervening nights.

The PN bag for home use is usually made with extra volume/'overage' in case the child's requirements increase.

In smaller infants, the rate of infusion should be reduced/wound down for the final hour or so (usually 50% of the full hourly rate for 30 min and 25% rate for the final 30 min prior to disconnection) in order to minimise the risk of rebound hypoglycaemia when the infusion is stopped.

When a child on PN at home is clinically stable and has at least some enteral tolerance, an attempt should be made to increase enteral intake and reduce PN. Points to consider when weaning PN at home are [18]:

- In order to improve quality of life, the PN should be reduced to the minimum possible number of nights/week at the earliest opportunity.
- If the child only tolerates a liquid enteral feed via an infusion rather than drinking it and is unlikely to gain enteral autonomy, the hours of infusion should be limited to just one or two per day.
- As soon as an infant can tolerate about 50% of requirements enterally, PN can be reduced to a maximum of five nights and possibly less.
- Rather than going straight to a night off PN, the parents/carers can halve the infusion volume one night a week and check if the child is well the next day. If successful they can stop the PN one night, the following week.
- If a night without PN can be tolerated, reduce the PN infusions to five nights/week with the two nights off spread through the week.
- If excess weight loss ensues when infusions are reduced, first increase the PN on the nights it is given, and review weight gain rather than immediately increasing the number of infusions.
- Enteral sodium supplements are often required the evening before and the morning after a night off PN.
- Growth and development should be monitored and PN adjusted as necessary. Dietetic input is essential to ensure earliest possible introduction of EN. Dietitians will usually contact families between face-to-face consultations when actively weaning.

When discharged, the child should be sufficiently stable to cope with the same PN formulation being given for a week, in order to give the homecare pharmacy a reasonable time to make changes to the formulation. If changes to the PN are required more frequently, the volume should be increased/reduced and supplemented enterally if extra electrolytes required. A portable infusion pump should be supplied to enable the child to be mobile in the evenings after connecting the PN. Many portable pumps have a rucksack to help support mobility when the child is connected to the infusion.

In certain circumstances, for example, when using a commercially available standard bag, the vitamins may be added at home. When patients have residual intestinal function, most vitamins (especially water soluble) can be adequately absorbed from the gastrointestinal tract.

The IF rehabilitation team should regularly audit the homecare service.

The family should have ease of access to contact the specialist centre by electronic messaging and phone calls. Face-to-face review by the specialist MDT intestinal rehabilitation service is usually only necessary on a 3-month basis when the child is stable [18].

Laboratory investigations/monitoring are also only needed 3 monthly when stable.

Appropriate investigations include blood urea, electrolytes, urine sodium, full blood count, vitamin A and E, ferritin, copper, zinc and selenium.

Vitamin D, manganese [13] and thyroid function should be checked annually. Other annual investigations include abdominal ultrasound to review the liver and kidneys and chest x-ray to assess position of the CVC (if in situ >12 months). Children aged over 5 years should have annual measurement of bone age and bone density [18].

The underlying intestinal disease predisposing the child to IF should be reviewed regularly and treatment appropriately adjusted.

Quality of Life in Children on PN at Home

Children who remain on PN into adult life can have a good quality of life [18]. Adolescents can do well at school and progress to higher education [44]. Children who remain on PN into adult life can gain employment [45].

Regaining Enteral Autonomy and Weaning PN Treatment

Weaning a child from PN to oral/enteral nutrition can be one of the most complex aspects of management. Even with extensive investigation and assessment of intestinal function, it is only certain whether a child has adequate intestinal function by reducing the PN and either allowing the child to eat or increasing infused liquid EN accordingly. Please see Table 46.7. Weaning from PN to EN at home usually takes place with:

- Dietetic support from the specialist unit.
- The main aim is to reduce the number of PN infusions/week.
- PN reduction can be done safely at home with support from the multidisciplinary IF rehabilitation team.

Transition to Adult Care

A small proportion of children remain on PN throughout childhood and need to transition from paediatric to adult care

on PN treatment [44]. Transition needs to be arranged on an individual basis since there are a limited number of patients on long-term care and each will have their individual needs. Although issues relating to transition are the same as for other chronic conditions, transfer needs to be performed between specialist IF units with the experience and resources to ensure the child complies with the process.

The two major changes are as follows: (1) the adolescent takes on 'ownership' of the condition, and (2) the parent relinquishes responsibility. The experienced units have the ability to provide a bespoke service for each patient with a professional (usually a specialist nurse) to provide support. The transition process can take 2 years for such complex patients.

Other treatment strategies : when long - term / home *PN* is inappropriate.

Intestinal Transplant

Despite a marked improvement in outcome in specialist IF rehabilitation centres with current PN management, there are still a small number of children who 'fail' PN treatment. Children who cannot continue with long-term PN and are not weaning from PN should be assessed for intestinal transplant. The indications for transplant are loss of vascular access, end-stage liver disease, unstable fluid balance and poor quality of life [27]. The transplant can range from small intestine alone to inclusion of the stomach and/or colon and if required the liver and other abdominal organs, multi-organ transplant. The outcome for long-term PN, despite the risk of potentially life-threatening complications such as CRBSI, can be up to 95% long-term/5-year survival [18, 46]. In contrast the 5-year survival for intestinal transplant according to the 2016 Scientific Registry of Transplant Recipients was 68% with 60% graft survival [47].

Withdrawing PN Treatment

Home PN was originally set up for otherwise healthy children with IF to be discharged home for a good quality of life. However, children with co-existing major organ failure are now referred for PN treatment at home. In some of these children, the burden of care is so great that long-term home treatment might prolong suffering when the child has no hope of eventual recovery and will never be capable of an independent life. In such situations ethical issues need to be addressed. For example, children with severe neurological impairment can develop intestinal dysmotility and hyperaesthesia with increasing age. The PN has often been started for what was thought to be an acute self-limiting illness that has progressed, and it has not been possible to wean the child off treatment. Early assessment by the hospital palliative care team should be arranged.

If it is agreed that a child with a life-limiting condition is started on PN, an 'end of life plan' should be discussed at an early stage. The plan is a guide, and parents can ask for it to be altered in a crisis if they change their minds. In other cases the child's condition may deteriorate when already on PN treatment. An MDT meeting can be held, and if appropriate a plan made for withdrawing PN treatment if it is considered inappropriate (for example appears to be causing rather than relieving suffering if continued). Psychosocial support and review should be made available.

Outcome

Management of PN is now so successful that a child can grow and develop normally on PN treatment throughout childhood and into adult life even when unable to tolerate little/no enteral nutrition.

In children with a primary digestive disorder, life expectancy is good. As long ago as 2012, a European survey reported a 2-year survival rate of 97%, 5-year survival rate of 89% and 10-year rate of 81% [46]. Outcome of different series varies according to the severity and diagnoses of patients discharged home [18].

Children can grow and develop normally on PN. IFALD usually improves in children discharged home. The number of intestinal transplants has been falling since 2008 [47] as IF rehabilitation management has improved.

Summary

A good nutritional state is a prerequisite for normal growth and development in childhood and a sense of well-being. The effects of inadequate nutrition in childhood may have lifelong consequences with stunted growth and intellectual development in addition to worsening any systemic illness. The child who develops IF today can expect to survive into adult life with a good quality of life with the support of PN managed by a multidisciplinary IF rehabilitation team.

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Intussusception

47

Rachael Essig, Brian A. Jones, and Mark B. Slidell

Introduction

Intussusception is a common cause of abdominal pain in children under 3 years of age. At the time of John Hunter's description of intussusception in 1793 [1], it was a nearly uniformly fatal diagnosis. In 1836 the very first therapeutic air enema was completed using a bellows followed in 1876 by Hirschsprung's development of a hydrostatic reduction technique [1, 4]. There were some improvements in patient outcomes following the introduction of these mechanical reduction techniques in the late 1800s; however, Frederick Treves still reported a case fatality rate greater than 70% in an operative series from 1885 [2, 3]. The first reports of fluoroscopic guided reduction with enemas were reported in 1927 [5], and the current success rate of radiologic reduction techniques should be greater than 80% to 90% in most centers [6, 7]. With advances in modern medicine, this disease is now consistently diagnosed and treated with minimal morbidity or mortality. In the current day and age, the mortality rate is <1% for both radiologic reduction and surgical intervention [8].

Incidence and Demographics

Intussusception is one of the most common sources of intestinal obstruction in children with an incidence of approximately 0.5 and 2 in 1000 infants and children [9]. The most common age of presentation is 3 months to 2 years with 40% of cases occurring between 3 and 9 months of age and 90% of cases occurring in children under the age of 3 years [10]. Intussusception is nearly twice as common in males as females [4, 9, 11].

Etiology

While overall 90% of all intussusception presentations have idiopathic etiologies, it is thought these are related to hypertrophy of the Peyer's patches in the small intestine from viral illnesses common in the pediatric population [12]. It has been noted 30% of pediatric patients presenting with intussusception had a prior viral illness [9].

The most common lead point for intussusception appears to be lymph node enlargement; however, it is uncommon to identify a pathologic lead point beyond typical lymphadenopathy secondary to inflammation. A pathologic lead point is estimated to occur only about 10% of the time, and this is typically in a child over 5 years old [9]. Extra-luminal processes such as intestinal duplication or Meckel's diverticulum can lead to intussusception as well as intra-luminal processes like polyps, inspissated feces, and parasites (Table 47.1) [9]. Older patients are more likely to have a pathologic lead point with up to 60% of children between the ages of 5 and 14 years old presenting with a lead point due to causes such as a Meckel's diverticulum, intestinal polyp, intestinal duplication, or lymphoma [13, 14].

Other rare causes of intussusception related to intestinal wall pathology include Crohn's disease, celiac disease, and intestinal allergies [9]. Intussusception may also occur in Henoch-Schonlein purpura secondary to submucosal

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Table 47.1 Pathogenesis and etiologies of intussusception

Pathogenesis	Idiopathic (95%)
	Pathologic lead point (4%)
	Post-operative (1%)
Infectious etiologies	Adenovirus
	Rotavirus
	Parasites
	Hypertrophied Peyer patch
	Lymphadenopathy
Anatomic etiologies	Appendix
	Crohn's disease
	Intestinal duplication
	Intestinal duplication
	Lipomas
	Lymphangioma
	Leiomyosarcoma
	Lymphomas
	Meckel diverticulum
	Peutz–Jeghers polyp or cancer
	Polyps
Other etiologies	Neuronal intestinal dysplasia
	Celiac disease
	Cystic fibrosis
	Bleeding disorders
	Henoch-Schonlein purpura
	Hemophilia
	Leukemia

hemorrhages that focally alter the normal peristalsis of the intestine leading to an aperistaltic lead point [9]. Intestinal pseudo-obstruction is another incompletely understood phenomenon in which a pseudo-obstruction of the small intestine may occur without any defined lesion within the intestine [9]. There are also iatrogenic sources of intussusception with the most common occurring around feeding tubes, such as a jejunostomy tube within the small bowel [4].

Pathophysiology

The pathophysiology of intussusception is that a lead point causes a portion of the intestine, known as the intussusceptum to invaginate and telescope into a distal segment of bowel called the intussuscipiens (see Fig. 47.1) [15].

This leads to an obstruction of the intestinal lumen. Eventually, with time the natural progression of intussusception leads to intestinal necrosis. Necrosis is caused by pressure on the mesentery of the intussusceptum by the intussuscipiens which leads to an obstruction of venous outflow. The ensuing edema in the intussusceptum peaks at the apex [15] of this segment of the bowel. An obstruction of venous outflow impairs arterial inflow leading to ischemia and eventual necrosis and perforation.

The most common type of intussusception in the pediatric age group is ileocolic, which occurs 80% of the time [16]. In this type of intussusception, the terminal ileum invaginates into the colon. Other types of intussusception are small bowel to small bowel and colo-colonic. Most of the pediatric literature on intussusception focuses on the management of ileocolic intussusceptions. Studies demonstrate that spontaneous small bowel to small bowel intussusceptions are quite common and rarely involve a lead point. These will spontaneously reduce over a brief period of time and may be found incidentally on a CT scan without cause for concern [17]. Certain congenital anatomic configurations are thought to predispose patients to intussusception. These configurations include a terminal ileum that inserts anterior onto the cecum, a cecum with immature taenia coli leading to increased flexibility, and lastly underdeveloped function of the colonic longitudinal muscle filaments near the ileocecal valve [9, 18]. Observation of these anatomical variations were seen in postmortem examination of autopsy specimens; it was determined that the process of intussusception occurs when the muscular ileocecal valve invaginates into a flaccid cecum due to lack of mature taenia coli [9, 18]. Another interesting anatomical variation that results in an increased risk of intussusception is an anomaly of intestinal rotation called Waugh syndrome. In this condition, the ascending colon lacks the usual lateral retroperitoneal attachments allowing the ileocecal valve to more freely prolapse and into the rectum [9]. Normally this can lead to an extensive intussusception and without significant intestinal ischemia due to the lack of colonic fixation. It is important to remember this as a possible presentation of malrotation so that the appropriate treatment for an anomaly of intestinal rotation is performed at the time of surgery [9].

Clinical Presentation

Intussusception is a common abdominal pediatric emergency. Parents can describe an acute onset of intermittent abdominal pain. During the episodic pain, children will often move their knees to their chest and demonstrate intense irritability [9]. In between these episodes, the child will frequently return to a normal level of activity, but with progression of the disease, the pain becomes increasingly stronger. It is not uncommon for associated nausea and vomiting to occur with the onset of the pain. Almost 50% of cases will present with the classic "currant jelly" bowel movements caused by the admixture of stool and blood [9]. In patients who undergo successful reduction of an ileocolic or colo-colonic intussusception, a recurrence may occur in 7.5% to 15% of patients within a brief time period following their original event [5]. Patients that have a recurrence of intussusception may present with more subtle signs the second time around, and this is why it is important to remember that a recent episode of intussusception places the patient at

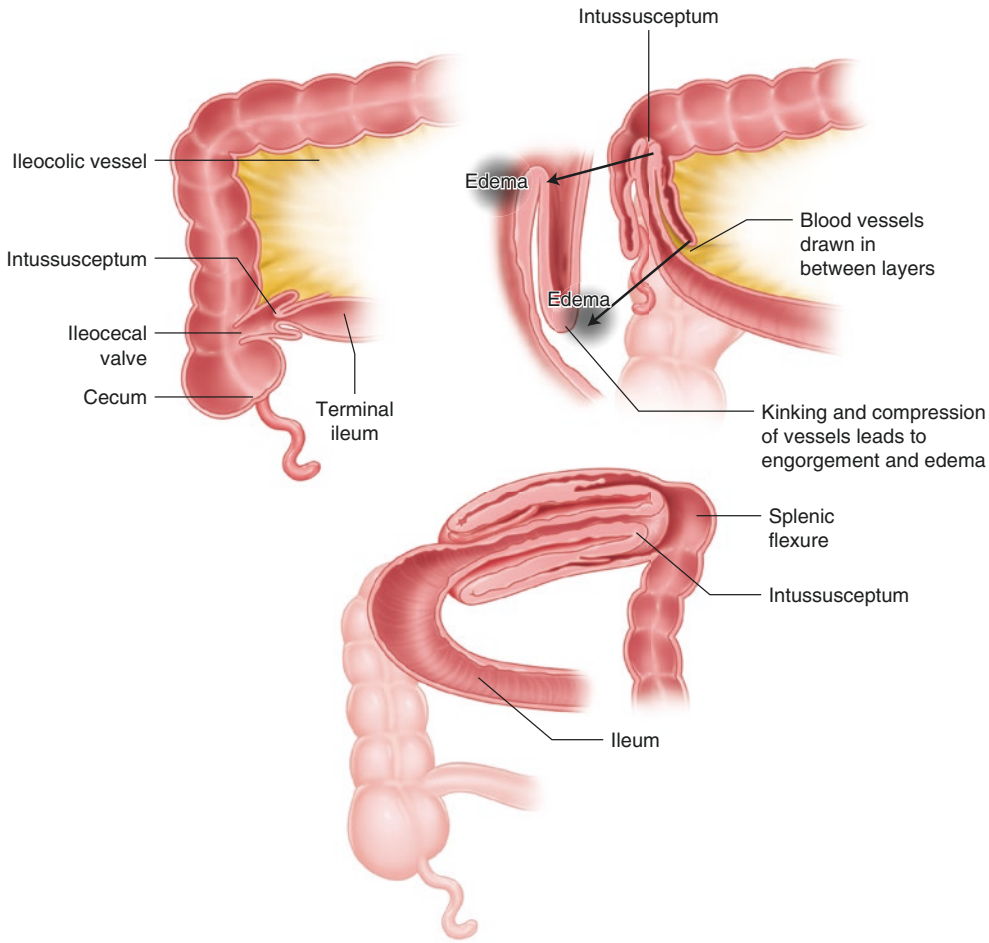

Fig. 47.1 Scheme of intussusception. Reproduced with permission. (This figure was published in Pediatric Surgery, Vo. 2, by Arnold Coran N. Scott Adzick Thomas Krummel Jean-Martin Laberge Robert Shamberger Anthony Caldamone, Intussusception, page 1094, Elsevier/Saunders, 2012)

higher risk of having a recurrent episode [19]. Early recurrence (<24–48 hours) rates range from 0.6 to 2.45% in most studies, and typically, 75% of recurrences will present within 6 months of the original event [19–23]. It is important to always keep a broad differential diagnosis list when initially evaluating a child with abdominal pain.

Diagnostic Evaluation

Suspicion for intussusception will depend to a degree on the child's clinical presentation. If there is a concern for peritonitis, an initial plain film chest or abdominal x-ray (Fig. 47.2) is an efficient diagnostic tool to establish a diagnosis of pneumoperitoneum; otherwise, a plain abdominal film has a 45% sensitivity to diagnosis intussusception [24, 25].

Abdominal ultrasound is a safe and quick diagnostic tool that may differentiate intussusception from numerous other etiologies of abdominal pain. Sonographic intussusception signs (Fig. 47.3) include the target (transverse view), doughnut (transverse view), or pseudokidney (longitudinal view) [11, 15, 16]. For an experienced ultrasonographer, the sensitivity and specificity approaches between 98–100% and 88–100%, respectively [24, 26]. Even in less experienced hands, ultrasound has been found very effective at diagnosing intussusception [27]. Benefits of ultrasound include the ability to differentiate the type of intussusception and identify potential lead points [17]. It is important to remember that up to 17% intussusception cases are transient and self-limiting [28]. Characteristics of transient, non-pathologic episodes of intussusception are that the segment of the involved bowel typically measures <3.5 cm in length and patients are asymptomatic [28].

Observation of decreased Doppler flow on ultrasound raises concern for intestinal necrosis. While color Doppler flow is not a perfect measure of intestinal necrosis, decreased flow is associated with lower rates of successful reduction by enema. The likelihood of reduction with air enema may vary from 94% in patients with visible blood flow to 31% without flow [29]. Nonetheless, in the absence of pneumoperitoneum, a finding of decreased flow on Doppler ultrasound does not preclude an attempt at reduction via contrast enema (see Fig. 47.4).

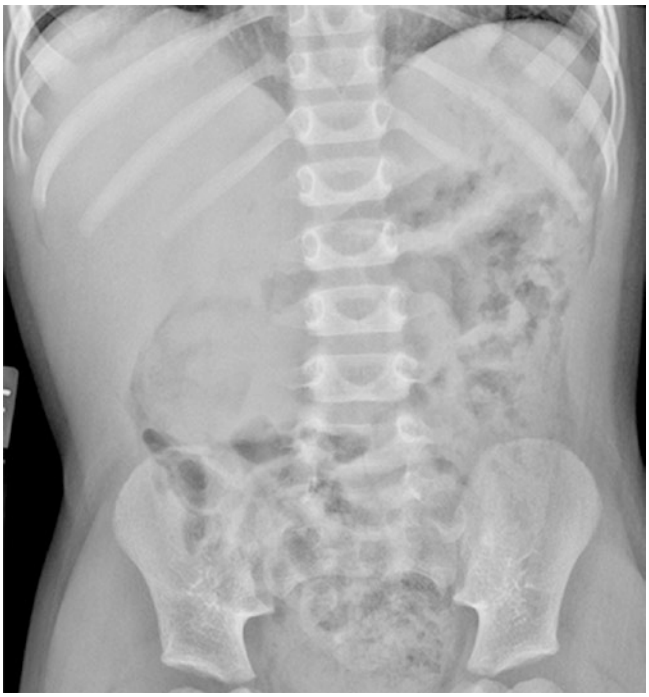

Fig. 47.2 Abdominal radiograph revealing a curvilinear mass in the right upper quadrant, consistent with possible intussusception

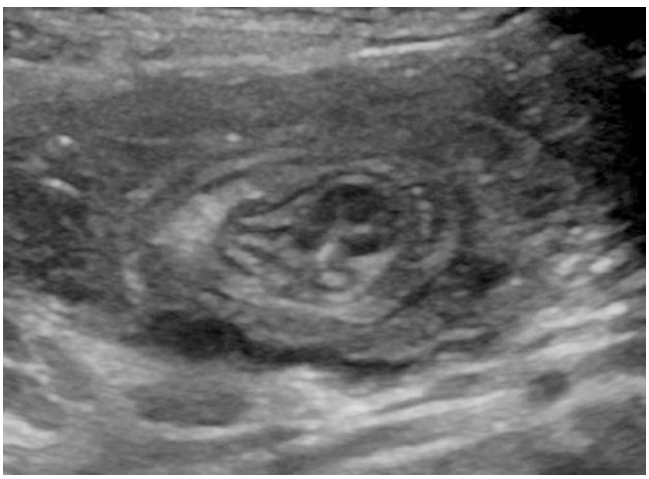

Fig. 47.3 Ultrasound is the primary imaging modality for the diagnosis of intussusception. In this image one can see a cross-sectional slice through the bowel demonstrating the "donut" or "target" signs on this transverse image. A "pseudokidney" sign may be seen on a longitudinal image

Clinical Management

On presentation with intussusception, it is important to evaluate the patient for signs and symptoms of dehydration as losses from both emesis and diarrhea can be profound. Dehydration and electrolyte abnormalities should be aggressively corrected while initiating diagnostic workup clinical stabilization may be necessary prior to any interventions. Occasionally these patients present with such severe electrolyte derangements that they require resuscitation in a pediatric intensive care unit.

Fig. 47.4 Contrast enema of the same patients as in Fig. 47.2, showing the presence of intussusception in the right upper quadrant. One can see the intussusceptum extending into the ascending colon up to the level of the hepatic flexure. The ascending colon is the recipient bowel (intussuscipiens) that contains the intussusceptum

Air or hydrostatic enemas are generally performed under fluoroscopic guidance by radiologists and in most cases are the first-line treatment for intussusception. Recurrence rates following a successful reduction are similar regardless of enema type employed [30]. Pneumoperitoneum, pneumatosis, and true peritonitis are a contraindication to enema reduction requiring prompt surgical intervention, while shock or dehydration is relative contraindications [5]. Aggressive fluid resuscitation may correct dehydration and shock, and then it may be safe to proceed with an attempt at enema reduction. Risk factors associated with unsuccessful of enema reduction are the presence of hematochezia, younger age, concurrent signs of intestinal obstruction, and

extended prodrome of symptoms [5, 31]. The most severe complication of an air enema is perforation of the intestine, which is rare and estimated to occur in 0.16–0.39% of procedures [5]. The risk of perforation is higher in children less than 6 months in age, those with an extended symptom prodrome, and those with unrecognized intestinal necrosis [5, 32]. Perforation may be at the site of intestinal necrosis or distally in the intestinal tract due to elevated pressure from the reduction. Tension pneumoperitoneum is an exceedingly rare complication of bowel perforation during an air enema [32]. This can be identified on physical exam or on ultrasound as extra-intestinal air or a Rigler's sign via fluoroscopy [33]. Treatment of this condition requires immediate cessation of the enema and, if hemodynamic instability is present, needle decompression of the abdomen [5, 33].

To ensure complete reduction of the intussusception, one should see air reflux into the small bowel and the resolution of the mass on imaging [34, 35]. Usually the radiologist will complete an ultrasound immediately after enema to confirm complete reduction. The overall success rate is relatively high between 80 and 95%. Historically, a successful reduction would require that the patient be monitored in a clinical setting for approximately 24 hours as 33% of recurrences occur during that time frame [19]. Recent studies have shown that patients successfully treated with an enema reduction of the intussusception may be safely discharged home after a brief period of observation in the emergency department. It is unclear if certain patients are at greater risk for recurrent intussusception; however, a recent retrospective study of 360 consecutive patients with successful air enema reduction found that age greater than 2 years was the only predictor of early recurrence [36], suggesting that children over 2 years of age might be safely discharged home from the emergency room after a brief period of observation and a successful PO challenge.

The air enema may be repeated if evidence of recurrence or incomplete reduction [34]. Repeated attempts at reductions, even if delayed, have been found to be safe if there is a partial reduction on the initial attempt and the patient continues to be clinically stable [5, 32]. It is hypothesized that the partial reduction of the initial enema allows for restoration of the venous drainage and enables a decrease in edema making subsequent attempts potentially more feasible [32]. Even an attempt at an air reduction enema is recommended first for intussusceptions caused by known lead points if there is no contraindication based on the patient's presentation, allowing emergencies to be handled on an urgent basis via surgery if reduction is successful [32]. For hospitals, without pediatric radiology expertise in enema reductions, a failed attempt at enema reduction for an ileo-colonic intussusception should not always lead directly to surgical intervention. If the radiologist is comfortable with a second repeat attempt at reduction after a delay of 45 to 60 minutes, then this is an option. Otherwise, consideration should be given to transferring the child to a regional children's hospital. There is good evidence that failure at a referring hospital does not preclude a repeat attempt at a children's hospital, and the success rate for a repeat attempt at reduction at the children's hospital should be approximately 60% [37].

For practitioners or families concerned about radiation exposure, it has been found that skilled radiologists are able to exclude intussusception with a fluoroscopy time of only 5–10 seconds [34]. Additionally, the reduction of a straightforward intussusception can occur in a short 14–30 seconds [34]. Air enemas appear to be superior to hydrostatic enemas due to the ease of air movement compared to liquids causing faster reduction rates and thus less radiation time. Another potential benefit to air reduction is the ability to maintain intra-intestinal pressure due to the nature of the valve inhibiting reversal of flow producing a better reduction rate. If a perforation occurs, there is potentially less morbidity with air compared to hydrostatic enemas likely due to decreased fecal contamination [5, 16, 34].

Surgical intervention is considered the first-line therapy when patients present with overt peritonitis on examination or pneumoperitoneum on x-ray [29]. Patients that have a delayed presentation are more likely to require eventual surgical intervention for management [17]. There are differing opinions regarding surgery as the first intervention if there is a pathologic lead point identified on imaging; likely this will be a multidisciplinary discussion between surgery, radiology, and the family about the risk and benefit of air enema reduction and surgery. Surgery can also be considered in same-site recurrent intussusception in the hopes of reducing further recurrences and the potential need for emergent surgical intervention [19].

Conclusion

Intussusception is a common cause for abdominal pain in the pediatric population. Efficient diagnosis and management with enemas have allowed for better outcomes with less invasive procedures for patients. Always keep in mind if there is any concern for a pathologic lead point or if this is a recurrent episode to involve pediatric general surgery.

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Meckel's Diverticulum

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Meghna S. Vaghani and Ashish P. Desai

Introduction

As Charles Mayo described, 'Meckel's diverticulum (MD) is frequently suspected, often looked for, and seldom found' [1].

The first descriptions of MD were by Heladnus in 1598 [2] followed by Levator in 1671 and Ruysch in 1730; much before Meckel's description [3]. Johann Friedrich Meckel is however credited with the first detailed description of the diverticulum and its embryonic origin in 1809 [4, 5].

In 1962, Harper et al. suggested that since 99mTc pertechnetate is concentrated by gastric mucosa, MD containing gastric mucosa should be identifiable scintigraphically using 99mTc pertechnetate [6]. Most MD may in fact be identified intra-operatively due to its varying presentation of intestinal obstruction caused by either band with volvulus or intussusception, umbilical discharge and diverticulitis.

Embryology

During the first few weeks of gestation, the primitive yolk sac divides into the primitive midgut and a smaller yolk sac. An omphalo-mesenteric duct, also known as the vitello-intestinal duct, connects these two structures and usually regresses between the 5th and 7th week of intrauterine life. Failure of regression leads to various presentations of patent vitello-intestinal duct (VI duct). Persistence of the tube only proximally causes MD.

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Epidemiology

Common dictum suggests MD is present in around 0.3–2.9% of the population. Whilst these figures offer guidance, incidence of symptomatic MD has been shown to decrease with age. It is thought to be around 4% in the first 3 years of life, and following that, the incidence decreases. The prevalence of MD during postnatal autopsies is 1.23% [7]. The majority of cases of symptomatic or resected MD occur in males, with systematic review data suggesting a male-to-female ratio of 1.5:1 to 4:1 [8].

Associated Anomalies

MD is usually an isolated condition; however patients with Hirschsprung's disease, Down syndrome, oesophageal atresia, duodenal atresia, malrotation and congenital cardiac abnormalities show increased incidence [9].

Gross and Microscopic Anatomy

MD arises on the anti-mesenteric border of the ileum and is approximately 2 inches long. Its distance from I/C valve is variable and is thought to be anywhere from 30 to 120 cm. Hence, during surgery, it is advisable to inspect at least 150 cm of the terminal ileum. It has its own blood supply from the persistent vitelline artery which is a branch of superior mesenteric artery.

Histologically, MD usually resembles the ileum. As it is a true diverticulum, it contains all layers of a normal intestine: mucosa, submucosa, muscularis propria and serosa with its own blood supply. However, it may contain heterotopic mucosa: either gastric or pancreatic tissue. Incidence of gastric mucosa occurs in around 9% of the patients, and pancreatic mucosa is seen in 2.5% of patients [10]. Various studies have also showed the presence of carcinoid, lipoma, leiomyoma or enterolith.

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Variations of Patent VI Duct Anomalies

VI duct anomalies occur due to variation in persistence of the VI duct remnant and its attachment to the umbilicus.

Various anomalies are:

1. MD – persistent proximal part of the VI duct.
2. Isolated fibrous band – persistent remnant of whole VI duct.
3. Omphalo-mesenteric fistula – presents as stoma at the umbilicus [11] or prolapse through the umbilicus [12].
4. Enterocyst – persistent middle part of the fistula.
5. Meckel's diverticulum may also have attached fibrous band.

Clinical Features

Most MDs are asymptomatic throughout life. In such cases MD may only be discovered incidentally during other diagnostic or surgical procedures. The clinical features of symptomatic MD can be summarised by the rule of 2: 2% of the population, within 2 feet of the I/C valve, and 2 inches long, two types of heterotopic mucosa [13].

Age-related presentations are further discussed below:

Neonatal

In neonates, presentation is usually due to persistent VI duct presenting as either a discharging sinus at the umbilicus [11] or a prolapsing VI duct remnant [14, 15]. Other presentations may be due to a band with associated volvulus causing partial or complete obstruction. Perforation is rare.

Paediatric Age Group

In the paediatric age group, presentation varies between intestinal obstruction (47%), gastrointestinal (GI) bleeding (25.3%) and diverticulitis (19.5%) [8]. Studies have shown that over 50% of symptomatic patients requiring surgical resection are under the age of 10 [16, 17].

Intestinal Obstruction

Obstruction is the most common presentation in children. Obstruction could be due to various reasons such as volvulus around the MD with a fibrous band, intussusception [18], enterolith, incarceration in Littre's hernia [19] or within umbilical hernia [20].

GI Bleeding

GI bleeding is the second most common presentation in children and the most common below 2 years of age. It is usually a major lower GI bleed, which is fresh and painless or associated with minimal pain. The majority of cases of bleeding are associated with gastric heterotopia [21]. The bleeding is due to a peptic ulcer in the surrounding normal ileal tissue. In cases where the gastric mucosa is absent, bleeding is usually attributable to other causes such as intussusception or gastritis.

Management of a major GI bleed should include assessment and treatment to achieve haemodynamic stability. This includes appropriate monitoring and fluid bolus. This should be followed by trying to differentiate between upper and lower GI bleeding, determining the site of bleeding and the diagnosis.

Children suspected to have MD should be started on H2 blockers such as ranitidine or famotidine after adequate fluid resuscitation. Currently, proton pump inhibitors such as omeprazole or lansoprazole are routinely used. Once the bleeding is controlled, a 99m technetium-pertechnetate scan should be carried out to look for the diagnosis of MD. If diagnosed, they should be resected.

Diverticulitis

Inflammation of the MD may be the presenting sign in 19.5% of children. They are often mistaken as acute appendicitis, and the children are usually older. During surgery, inflamed MD is identified and should be excised.

Adult Age Group

As discussed, the incidence if symptomatic MD decreases with age. If MD does present in adulthood, obstruction and diverticulitis are the more commonly seen [8]. An increased incidence of MD-related cancers is also seen in adults with a mean age of 60.6 years. Furthermore, adjusted risk analysis shows a 70 times higher likelihood of MD malignancy than any other ileal site [22].

Other Presentations

Other rare presentations can be due to development of bezoar in the diverticulum [23] or malignancy. The most common tumour affecting the MD is carcinoid tumour [24, 25]. However, villous adenoma [26] or GI stromal tumour [27] has also been reported.

Management of MD

Investigations

As MD has varied presentations, it is not always possible to diagnose it prior to surgery. The surgeon needs to have high index of suspicion.

The investigations which might raise the possibility of MD are as follows:

1. *Plain X-ray of the abdomen:* Plain X-ray may show evidence of intestinal obstruction. If enterolith is present, it may be visible. If MD has perforated, pneumoperitoneum may be visible.
2. *Scintigraphy:* Harden et al. demonstrated that technetium-99m was concentrated in the gastric mucosa. It can therefore demonstrate the presence of heterotopic gastric mucosa in MD when present. The isotope is selectively taken into gastric, salivary and thyroid tissue and excreted in urine. Hence, false-negative scans may be reported if the diverticulum is overlapped by the urinary bladder. Sensitivity of the scan can be increased by using either pentagastrin or glycogen [28]. These scans have specificity of more than 95% and sensitivity of more than 85%.
3. *Fistulogram:* Patent VI duct may be identified by fistulogram from umbilical fistula.
4. *GI contrast study:* It is unusual to identify MD by barium meal. However, it may be visible in 0.7% cases by small bowel enema.
5. *Angiography:* In adults and older children, bleeding MD may be identified by selective superior mesenteric artery angiography. If the patient is very unstable, it may be possible to temporarily occlude the bleeder by embolising the vessel. The patient should have laparotomy as soon as they are stabilised as embolisation may lead to gangrene and perforation of MD in a few hours' span.
6. *Capsule endoscopy:* Whilst endoscopic patterns for MD have not yet been defined, research suggests that the presence of a double lumen with or without bleeding and/or a diaphragm sign may suggest MD. Localisation in the lower right quadrant is likely MD, though localisations in other areas may need further investigation [29, 30].

Incidental MD

In the event of an incidental MD during laparotomy, the decision to excise will be evaluated based on multiple factors such as cause for the surgery, length and breadth of the MD base and presence or absence of a palpable nodule suggestive of heterotopic mucosa.

Surgical Management

In symptomatic cases, resection of the diverticulum is usually recommended. The whole diverticulum with heterotopic mucosa, if present, should be excised. After the excision, the bowel needs to be anastomosed without causing luminal obstruction. This can be achieved either by diverticulectomy alone if the base is narrow or by resection of the ileum containing the diverticulum with end-to-end anastomosis.

The decision between diverticulectomy and segmental resection is usually evaluated by the surgeon. The MD Resection Index (MDRI) has been proposed as a method for this evaluation and is calculated using the length and width of the diverticulum [31].

This procedure was routinely performed by laparotomy, but with changing practice, laparoscopy, standard three-port or using single-port technique [32], is playing an increasing role. During diagnostic laparoscopies performed for GI bleeding or acute appendicitis, routine examination of the terminal ileum should be carried out for at least 150 cm from the I/C valve to look for MD. The surgeon should start inspecting the ileum systematically from the I/C valve. Two bowel graspers at anti-mesenteric border should grasp intestinal loops, and both sides should be examined. When MD is identified, it can either be excised in situ, or the same can be delivered outside the abdominal cavity via the umbilical port and the resection and anastomosis performed. The intestine can then be reposited inside.

Outcome/Conclusion

Meckel's diverticulectomy has low morbidity and mortality. However, its first presentation may be at any age and is varied with obstruction, diverticulitis, haemorrhage or neoplasm [33]. Hence, the clinician needs to show a high degree of suspicion and look out for this. It is seldom found easily; therefore early diagnosis and proper treatment are essential to manage its varied clinical symptoms. Surgical and laparoscopic techniques have developed over time, and today laparoscopy is increasingly being used in the management of MD.

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Appendicitis

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Megan E. Bouchard, Mark B. Slidell, and Brian A. Jones

Diagnosis of appendicitis is usually not especially difficult.
William Silen – Cope's Early Diagnosis of the Acute Abdomen 1979

I doubt whether there is any disease which has symptoms of such variable degree as appendicitis. Everyone has seen children with negligible symptoms who, at operation, have had gangrene of the appendix. It is these mild cases so easily missed, which eventually make every clinician of experience eat his portion of fricasseed crow.
Willis J. Potts – The Surgeon and the Child 1959

Introduction

Appendicitis, or inflammation of the appendix, is the most common emergent surgical condition in children and accounts for approximately 10% of all pediatric emergency room visits [1]. While some considered it a vestigial organ, the appendix serves as a reservoir for normal intestinal microbiota and has the highest concentration of gut-associated lymphoid tissue in the intestine [1]. Despite its high incidence, appendicitis is the most frequently misdiagnosed surgical condition of the abdomen [2]. To minimize misdiagnosis and standardize care, several algorithms to both diagnose and treat appendicitis have been developed for children.

Classically, appendicitis is subdivided into (1) acute, simple appendicitis; (2) acute, complicated appendicitis; and (3) chronic appendicitis [3]. Simple appendicitis is a process that is confined to the appendix [1, 3]. Complicated, or sometimes referred to as complex, appendicitis instead indicates more advanced pathology including appendiceal perforation, phlegmonous or gangrenous changes, or an associated abscess [1, 3]. Chronic appendicitis is less common and remains a controversial topic [4]. The literature suggests this is a real entity defined by inflammation of the appendix that lasts for weeks, months, or even years [4].

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Epidemiology

The annual incidence of appendicitis is estimated at 19–28 per 10,000 children [5]. Appendicitis is more commonly diagnosed in children aged 4–14 years old [5, 6]. Children less than 4 years old have a lower annual incidence of one to six per 10,000 children per year [7]. Overall, the estimated lifetime risk of developing appendicitis is 7–8% [6, 8]. Appendicitis has a male predominance of about 55–60% [8]. The percentage of children who present with complicated appendicitis is estimated at 30%, ranging from 20% to 74% depending on the study [5, 8–11].

Higher rates of complicated appendicitis have been seen in patients with greater than 48 h' duration of symptoms, age less than 5 years old, rural geography, ethnoracial minority, public or self-insurance, obesity, and other chronic diseases [10–12]. Complicated appendicitis is also associated with increased length of stay, complications, and hospital cost [8, 11].

Anatomy and Pathophysiology

The appendix is a blind-ending luminal structure attached to the base of the cecum near the ileocecal valve. The base of the appendix is most commonly located at McBurney's point, which is located one-third the distance from the right anterior superior iliac spine to the umbilicus. The tip of the appendix, however, can be located in various positions including pelvic, subcecal, retroileal, retrocecal, ectopic, and preileal locations. While it is most commonly positioned in the pelvis, the variations in location may affect the presenting symptoms of appendicitis and complicate the diagnosis. The appendicular

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artery, a branch of the ileocecal artery, is the blood supply of the appendix. The appendix is composed of colonic epithelium, with the submucosa containing a high concentration of lymphoid follicles, most especially in children.

Acute appendicitis is caused by obstruction of the lumen of the appendix [13]. Once obstructed, the mucosa continues to secrete mucus and fluid leading to increased pressure within the obstructed lumen [13]. The resident intestinal bacteria continue to grow and recruit neutrophils, which leads to the production of purulent fluid and even higher intraluminal pressure [13]. The high pressure causes obstruction of venous outflow and lymphatic drainage [13]. Ultimately, the arterial blood flow may also become obstructed from the significant edema, leading to transmural appendiceal ischemia [13]. Once the bacteria invade the appendiceal wall, the diseased appendix is then at high risk for perforation [13].

Most commonly caused by fecal stasis or a fecalith, obstruction of the appendix in children may also be caused by lymphoid hyperplasia from systemic infectious illness, granulomatous inflammatory changes with Crohn's disease, thick mucus seen in cystic fibrosis, parasites, or rarely appendiceal neoplasms [5, 6, 9]. Interestingly, a family history of appendicitis is associated with a nearly threefold increased risk of developing appendicitis, suggesting there may also be a genetic component [14].

Presentation and Physical Exam

Presenting Symptoms

Classically, appendicitis begins with the onset of gradual, constant periumbilical pain that eventually migrates and localizes to the right lower quadrant [15]. While the migration of pain is highly specific, it only occurs in approximately half of cases [15]. The periumbilical pain is due to swelling of the appendiceal lumen causing irritation of the visceral peritoneum. The right lower quadrant pain is then due to direct irritation of the parietal peritoneum and ultimately results in somatic pain.

Other common symptoms of acute appendicitis include nausea (81.7%), emesis (67.7%), and anorexia (72.4%) [16]. While most patients are afebrile or simply report a low-grade fever, high fever is uncommon and raises suspicion for perforated appendicitis [17]. In children, the diagnosis of appendicitis can be challenging because they do not always have the classic signs of right lower quadrant pain, fevers, and nausea or anorexia [8, 17]. This is especially true among younger children who may have more difficulty explaining their symptoms and may instead present with irritability, decreased activity, and refusal to eat [8, 17].

In pediatric appendicitis, approximately 50% of patients present with atypical features including normal or increased bowel sounds (64%), absence of rebound pain (52%), lack of migration of pain (50%), lack of guarding (47%), lack of anorexia (40%), and absence of maximal pain in the right lower quadrant (32%) [17]. Given many patients present with these atypical features and the classic symptoms incidentally overlap with presentations of many other common causes of childhood abdominal pain, the diagnosis of pediatric appendicitis can be quite challenging [8, 17].

Physical Exam

On physical exam, it is important to not overlook vitals and the general appearance of the patient. Observations of tachycardia, fever, acute distress, listlessness, and irritability may all be important in establishing the diagnosis. Beyond the complete general physical exam including examination of the lungs, heart, periphery, and ear/nose/throat, the primary exam will be focused on the abdomen.

Beginning with inspection, the abdomen may or may not be distended. If the patient is old enough to participate in the exam, it can be helpful to ask the patient to point to where they feel the most pain. Next, palpate the abdomen in each quadrant, asking the patient to indicate if they experience any pain and where. Observing facial expressions during palpation can also be useful. Classically, patients will exhibit maximal tenderness at McBurney's point, though variations in the location of the appendiceal tip or complications from perforated appendicitis may result in pelvic or diffuse pain. Patients with peritonitis typically lie still and are reluctant to change positions. Gentle percussion or deep palpation with a quick release may elicit rebound tenderness and also indicate peritonitis. Less commonly, a mass may be palpated in the right lower quadrant with more advanced disease.

Classic adjunct maneuvers include the Rovsing, obturator, and psoas signs. The Rovsing sign is positive when palpation of the left lower quadrant results in right lower quadrant pain. The obturator sign is positive when pain is elicited with flexion and internal rotation of the right hip and increases suspicion for pelvic appendicitis. Finally, the psoas sign is positive when the patient is placed in left lateral decubitus position and right hip extension elicits pain; this sign increases suspicion for retrocecal appendicitis. While these physical exam maneuvers may increase the likelihood of appendicitis if positive, labs and imaging are often ordered to differentiate between appendicitis and other diagnoses with similar signs and symptoms.

Diagnosis

Differential Diagnosis

The differential diagnosis for pediatric conditions with overlapping signs and symptoms of appendicitis is broad and var-

ies by sex and age. Other gastrointestinal pathologies include gastroenteritis, Meckel's diverticulitis, intussusception, inflammatory bowel disease, typhlitis, mesenteric adenitis, malignancy, bowel obstruction, and constipation. In girls, it is important to consider pelvic inflammatory disease, ectopic pregnancy, dysmenorrhea, ovarian torsion, and ovarian/paratubal cysts. Other conditions to consider include right lower lobe pneumonia, nephrolithiasis, pyelonephritis, cystitis, pancreatitis, lymphoma, Henoch-Schonlein purpura, and hemolytic-uremic syndrome.

Diagnostics: Laboratory

There is no definitive algorithm for work-up of pediatric abdominal pain concerning for appendicitis, though some diagnostics are important to consider. First, obtaining a complete blood count (CBC) to evaluate the white blood cell count can be helpful. After the first 24 h of symptoms, the CBC may demonstrate a left shift with a predominance of neutrophils and bands [18]. While leukocytosis is a nonspecific marker of inflammation, the increase in neutrophils would increase the concern for infection [18]. It is important to note that leukopenia, rather than leukocytosis, may be present in those who are immunocompromised and may raise the concern for other conditions such as typhlitis [19]. Additionally, a urinalysis should be sent to rule out urinary tract infection and a pregnancy test sent for girls of reproductive age. If the patient reports a significant history of recent emesis and/or diarrhea, obtaining a basic metabolic panel will be helpful in evaluating for electrolyte derangements and acute kidney injury from dehydration. Children with complicated appendicitis will often present with diarrhea secondary to purulent fluid in the abdomen or pelvis causing inflammation of the intestines [20]. In those patients that report pain more in the periumbilical or right upper quadrant regions, it would also be useful to obtain liver function tests and a lipase to rule out hepatobiliary and pancreatic pathology.

Diagnostics: Imaging

Imaging can assist with obtaining a diagnosis, reducing negative appendectomy rates and reducing lengths of stay [21]. In pediatrics, the standard imaging to begin with in diagnosing appendicitis is an abdominal ultrasound, though obtaining quality ultrasound images is user dependent [22]. The advantages of ultrasound are that it is fast, portable, and less expensive than other imaging modalities and delivers no radiation to the child. Nevertheless, visualization of the appendix may be limited by the patient's body habitus, bowel gas, abnormal location of the appendix, or severe pain that hinders abdominal compression with the ultrasound [22]. Despite these limitations, the sensitivity and specificity of diagnosing appendicitis on ultrasound is as high as 90–95% [23].

On ultrasound, an inflamed appendix in children should be greater than 6 mm in diameter and have a wall thickness greater than 3 mm [22, 24]. Other signs that may increase the suspicion for appendicitis include periappendiceal lymphadenopathy and free fluid, hyperechogenic pericecal fat, increased blood flow on Doppler scan, visualization of an appendicolith, and presence of an abscess [22, 24]. On the axial plane, an inflamed appendix will have a target appearance and will be non-compressible [22, 24]. If appendicitis is not visualized, an ultrasound can also assess for other abdominal pathologies including ovarian cysts, ovarian torsion, hepatobiliary pathology, and intussusception.

An abdominal X-ray is not specific or sensitive for diagnosing appendicitis, though may rarely demonstrate a radiopaque appendicolith. Most patients with appendicitis will have a normal-appearing abdominal X-ray [25]. An abdominal X-ray may reveal other causes of abdominal pain that mimic appendicitis, including intussusception, typhlitis, bowel obstruction, ileus, and constipation.

In cases where ultrasound is inconclusive, some hospitals elect to obtain a focused abdominal MRI (magnetic resonance imaging) [26]. Similar to ultrasound, MRI delivers no radiation to the patient, though is not user dependent and provides high-quality images with high sensitivity for appendicitis [26]. Nevertheless, if a focused MRI protocol is not in place at the institution, the scan duration may be quite long, be delayed due to other uses, or require sedation.

Many institutions, especially non-pediatric centers, use abdominal CT (computerized tomography) to diagnose any abdominal pathology. Unlike ultrasound, a CT scan is not dependent on user accuracy. CT can diagnose abdominal pathology other than appendicitis, is fast, and has high sensitivity (94%) and specificity (95%) [16]. Unfortunately, CT imaging does expose the patient to radiation and is much more expensive than ultrasound.

Given all these factors, most pediatric centers would recommend beginning with an abdominal ultrasound to evaluate for appendicitis [8, 22]. Selective use of MRI to follow an inconclusive ultrasound can be effective in establishing the diagnosis with a sensitivity of 100% and specificity of 96% [27]. These findings accompanied with a physical exam consistent with appendicitis lead to a positive predictive value of 83% and a negative predictive value approaching 100% [8, 28]. This work-up pathway has not been shown to increase the risk of perforation or negative appendectomy rates [26]. The time to treatment and length of stay are also unchanged [26].

Scoring Systems to Evaluate for Appendicitis

Another tool that can help in determining the next step in the work-up of a patient with possible appendicitis scoring systems such as the Pediatric Appendicitis Score (PAS) (Table 49.1) [29, 30]. Based on identified signs, symptoms, and laboratory findings, the patient receives a total score from 1 to 10 [29]. The PAS score assesses for tenderness to palpation in the right lower quadrant, anorexia, low-grade fever (>38.0), nausea/emesis, leukocytosis (>10,000/mm^3), left shift (>75% neutrophils), migration of pain to the right lower quadrant, and cough/percussion/heel tapping tenderness in the right lower quadrant. Studies have demonstrated that using the score alone is not sufficient to diagnose appendicitis, but using it in tandem with imaging can risk stratify patients for suspected appendicitis, decrease the time to diagnosis, and limit radiation exposure [8, 29, 30]. In fact, false-negative ultrasound findings decrease with increasing PAS, and conversely false-positive ultrasound findings increase with decrease PAS [30]. There are other validated, risk stratification scoring systems such as the Alvarado score [31] and the Appendicitis Inflammatory Response (AIR) score [32]. All three systems have utility, and differences in the various components of the scoring systems illustrate the challenges in making a confident diagnosis of appendicitis in the emergency setting. None of these systems takes into account two other key factors such as the duration of symptoms or whether the pain is constant or intermittent in nature. We propose that a new scoring system incorporating these factors might have improved diagnostic yield.

In instances where the diagnosis remains unclear, patients can be admitted for observation and serial abdominal exams. In cases of diagnostic uncertainty, antibiotics are generally withheld as antibiotics may effectively teat early appendicitis and mask developing symptoms. Most patients' symptoms will either resolve or progress, and the diagnosis should become clear.

Table 49.1 Comparison of the Pediatric Appendicitis Score (PAS), Alvarado score, and the Appendicitis Inflammatory Response (AIR) score. All three are validated, risk stratification scoring systems, in common use by emergency medicine physicians. The Pediatric Appendicitis Score appears to be the most used system

		Scoring system		
		PAS	**Alvarado**	**AIR**
Symptoms				
	Nausea or vomiting	1	1	–
	Vomiting	–	–	1
	Anorexia	1	1	–
	Migration of pain to RLQ	2	1	–
Signs				
	Pain in RLQ	2	2	1
	Rebound tenderness	1	1	–
	Light	–	–	1
	Medium	–	–	2
	Strong	–	–	3
	Body temperature >37.5 °C	–	1	–
	Body temperature >38.5 °C	1	–	1
Laboratory tests				
	Leukocytosis shift	–	1	–
	PMN leukocytes (>75%)	1	–	–
	70%–84%	–	–	1
	>85%	–	–	2
	WBC			
	>10 × 109/L	1	2	–
	10.0–14.9 × 109/L	–	–	1
	>15.0 × 109/L	–	–	2
	CRP Concentration			
	10–49 g/L	–	–	1
	>50 g/L	–	–	2
Total score		10	10	12
Risk of appendicitis		**PAS score**	**Alvarado score**	**AIR score**
	Low-risk	1–4	1–4	0–4
	Intermediate-risk	5–7	5–6	5–8
	High-risk	8–10	7–10	9–10

Abbreviation: *RLQ* Right lower quadrant

When to Consult Surgery or Transfer the Patient to a Center with Pediatric Surgery

Whenever there is a concern for an acute abdomen, surgery should be consulted sooner rather than later. However, if the patient is clinically stable, there is time to begin the diagnostic work-up prior to consultation. Using the PAS tool can be useful to risk-stratify patients and to determine when to consult surgery [29, 30].

For patients with a PAS >6, surgical consultation is warranted, and a discussion with the surgeon regarding whether abdominal imaging is indicated [29, 30]. For patients with a PAS 4–6, it is often helpful to obtain cross-sectional imaging such as an ultrasound or MRI [29, 30]. If the appendix is not visualized on the imaging, then surgical consultation may be reasonable to decide on the disposition of the patient. If the PAS is <4, the patient has a low likelihood of appendicitis and does not necessarily warrant further imaging [29, 30]. This patient is less likely to benefit from a surgical consultation, and other causes of acute abdominal pain should then be considered.

If the patient is being seen in a hospital without pediatric surgery, the provider should consider transferring the patient after establishing the diagnosis or when there is any concern for a possible need for surgical intervention [33, 34].

Management

Initial Management of All Appendicitis Patients

Once the diagnosis of appendicitis is established, the most important first step in management is resuscitation and initiation of antibiotics [6, 8]. Fluid resuscitation may begin prior to completing the work-up, as most patients with appendicitis are dehydrated [6, 8]. The choice of antibiotics varies across institutions. Antibiotics should cover enteric flora including gram-negative and anaerobic bacteria. Providers should also consider the patient's allergy history and their hospital's local antibiogram and resistance patterns.

Surgical Management: Appendectomy

The gold standard treatment for appendicitis is appendectomy, either open or laparoscopic [1, 35]. Both approaches have good outcomes, though patients who undergo laparoscopic appendectomy report less pain, faster discharge, earlier mobilization, and early resolution of ileus [1, 35]. Additionally, laparoscopic appendectomies allow for a better cosmetic result and the ability to easily assess for other intraabdominal pathologies [1, 35]. If the appendix appears normal and no other pathology is identified, most surgeons will still remove the appendix to reduce the diagnostic conundrum if the symptoms persist or return. There are very few contraindications to appendectomy, though patients presenting in septic shock should be resuscitated prior to undergoing surgery. The appendectomy should be performed with some degree of urgency (<24 h from presentation), though does not to be performed emergently (<2 h from presentation) [36].

Several studies support a shift away from the traditional thinking that appendicitis was an emergency and that surgery ought to be performed immediately at the time of diagnosis [37–39]. Children presenting with >48 h of symptoms are more likely to present with complicated appendicitis; however the length of time from diagnosis to the operating room appears to play little to no role in whether the appendix will "perforate" while they await the operating room [37–39]. A study of 230 children which examined the impact of the time from diagnosis to appendectomy supports this change in thinking [38]. Children taken to the operating room at 0–3 h, 4–6 h, or longer than 6 h after diagnosis of appendicitis were not found to have a statistically significant difference in perforation rates or hospital length of stay [39]. Appendectomies that are performed emergently are not associated with decreased rates of perforation or other complications relative to those performed up to 24 h after presentation [37, 38]. Additionally, there is evidence to suggest those children rushed to the operating room may have a higher rate of post-operative surgical site infection in non-perforated patients and that children operated on after overnight fluid resuscitation and antibiotics have a lower risk of post-operative abscess [35, 40]. While in-hospital surgical delay does not appear to increase the risk of finding complicated appendicitis at the time of surgery, delays in patient presentation to the hospital are associated with a 4.9 times increased odds of perforation and a 56% increase on hospital length of stay [40].

After appendectomy, it is important to send the appendix specimen to pathology for review. The diagnosis should be confirmed with histology that may demonstrate acute inflammatory infiltrate, necrosis, thrombosis, and possible transmural infarction in perforated appendicitis. Other pathologies, besides appendicitis, may also be seen. Occasionally, no pathology at all is visualized. The rate of negative appendectomies for pediatric patients is estimated at 3.6%, though is higher for children less than 5 years old and girls older than 10 years old [41]. Patients who undergo diagnostic imaging pre-operatively have lower rates of negative appendectomies, regardless of age or sex [21]. Balancing the risks of perforated appendicitis, a negative appendectomy rate of less than 5% is acceptable at most institutions.

Children with simple appendicitis can be discharged soon after surgery once they demonstrate adequate pain control and oral intake [42]. They do not require further antibiotics. Patients with complicated appendicitis are admitted and maintained on intravenous antibiotics. The total antibiotic duration, intravenous and oral, should be approximately 3–7 days, though varies by patient and provider [43]. Some patients experience post-operative ileus, so their diets are advanced more slowly and occasionally require nasogastric decompression [1]. In patients who are not receiving oral nutrition after 1 week, parenteral nutrition should be considered. Patients with complicated appendicitis are also at high risk for a post-operative inflammatory response and are likely to require ongoing resuscitation with close attention paid to their urine output [1, 6, 8]. Finally, complicated appendicitis significantly increases the risk of development of a post-operative intraabdominal abscess or wound infection [8, 44]. Fever on post-operative day 5 or later should include examination of the incisions and consideration of imaging to rule out a possible intra-abdominal infection. Most patients with complicated appendicitis will have resolution of their symptoms by post-operative day 5. In cases where there is concern for an abscess, waiting until post-operative day 7 before scanning a patient will lead to fewer drainage procedures and decrease the need for additional cross-sectional imaging [45]. Depending on the size and location, well-formed fluid collections can be managed by antibiotics, image-guided drainage, or operative drainage [45].

Nonoperative Management

Patients who present with large well-formed abscesses from perforated appendicitis may benefit from delay in appendectomy [46]. These patients have often had symptoms for several days prior to presentation. Management consists of resuscitation, intravenous antibiotics, and bowel rest (NPO). The abscess is then drained percutaneously if safely accessible and a drain left in place to allow for ongoing drainage [46]. Patients' diets are slowly advanced, and the patient is discharged home on antibiotics with or without the drain in place.

Recently, many researchers have demonstrated resolution of pediatric appendicitis with antibiotics alone [47]. Patients are admitted for observation, intravenous antibiotics, resuscitation, and bowel rest. As their pain improves, patients' diets can be slowly advanced. Once their pain is resolved, they are no longer having fevers, and they are tolerating a regular diet, patients can be discharged home to complete an oral course of antibiotics. However, if patients demonstrate new-onset hemodynamic instability, rising leukocytosis, worsening pain, or persistent fevers after 24 h, the conservative nonoperative management is deemed unsuccessful, and appendectomy should be reconsidered [48]. For patients with perforated appendicitis, conservative management fails in as many as 10–25% of children [48]. Additionally, patients with evidence of an appendicolith are at high risk for failure of conservative management at 72% [49]. In patients with simple appendicitis, conservative management is successful in 72.7–90% of cases in resolving symptoms [50–52]. Conservative management is associated with longer lengths of stay, repeat imaging, and prolonged antibiotics [47].

Interval appendectomy can be offered after conservative management of appendicitis. Interval appendectomies are typically performed 2–3 months after the initial bout of appendicitis. Some proponents of conservative management are beginning to question whether interval appendectomy is necessary [53]. Interval appendectomies decrease the chance of recurrent appendicitis and allow for the evaluation of alternative pathology, such as inflammatory bowel disease or a neoplasm that led to the appendicitis. However, others report the risk of recurrent appendicitis is low enough to avoid subjecting the patient to the anesthesia, surgical risk, and extra cost [53]. As the utility of interval appendectomies and conservative management of appendicitis continue to be debated, patient and family preference will be useful in determining treatment plans. Ultimately, it is important to practice shared decision-making and to recognize patient reported outcomes such as pain, quality of life, disability, general anesthesia avoidance, and acceptance of the risk of recurrent appendicitis [54].

Follow-Up and Post-Operative Complications

After discharge, patients may develop a wound infection (<2% for laparoscopic appendectomy and 3–11% for open appendectomy) or an intraabdominal abscess (5% for simple appendicitis and 15% for complicated appendicitis) [8, 55, 56]. Evidence of cellulitis around the incision sites, fever, or ongoing abdominal pain requires further evaluation and possible diagnostic studies.

While waiting for an interval appendectomy after conservative treatment, approximately 10% of patients will have recurrent appendicitis. For those who do not undergo interval appendectomy, the risk of recurrent appendicitis ranges from 5% to 37% within the first year from the initial appendicitis diagnosis [53, 57]. After that year, the risk returns back to the general population lifetime risk of 7% [1]. However, patients with evidence of appendicolith are at increased risk or recurrence and therefore should undergo interval appendectomy [58].

The majority of patients do very well with timely management of appendicitis. Overall, the mortality rate for pediatric appendicitis is 0.1–1% and most commonly occurs in neonates and infants, where appendicitis is less common and diagnosed later [5, 55].

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Gastrointestinal Vascular Anomalies

50

Melania Matcovici, Indre Zaparackaite, and Ashish P. Desai

Introduction

Vascular abnormalities are either present at birth or appear soon after. They are more commonly seen as cutaneous lesions.

The first scientific report of vascular malformations appeared in the literature in the seventeen century. In 1628, *William Harvey* published his major work, *On the Motion of the Heart and Blood in Animals*, where he explained blood circulation. Since then, there was a continuous scientific urge to explore the secrets of abnormal circulation and aberrant vascular communications.

In the nineteen century, a German scientist, *Rudolf Virchow* (1821–1902), the founder of cellular pathology, was the first to suggest that hemangioma is formed of new vessels rather than by passive dilatation of preexisting channels. He also speculated that these tumors appeared antenatally and continued to grow postnatally.

Embryology

The vascular system arises from mesodermal blood islands in the wall of yolk sack. These blood islands are composed of progenitors of blood cells and endothelial progenitor cells. Further development encompasses vasculogenesis (formation of embryonic vessels), migration of angioblasts to the organs, and angiogenesis (the process of growing new vessels). Angiogenesis is regulated by local factors maintaining the subtle balance between the inhibitors (e.g., thrombospondins, endostatin) and stimulators (e.g., vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and angiopoietins). VEGF is a key regulator in angiogenesis and has been found to be responsible for inducing penetration of the capillary vessels into the avascular epidermis [1]. As an end product, the vessels are composed of a single layer of endothelial cells surrounded by a variable number of layers of vascular smooth muscle cells.

Pathogenesis

The precise pathogenesis of development of vascular lesions is not fully understood. Nevertheless, development process should be considered separately for tumors and malformation.

Vascular Tumors

Vascular tumors are characterized by increased endothelial proliferation. The precise origin of the hemangiomal endothelial cells remains uncertain. Immunophenotypic similarities with placental endothelium suggest a placental origin [2]. The mechanism is believed to be embolization of placental endothelial cells, which enter the fetus from chorionic villi through right-to-left shunts (normal in fetal circulation). This theory was checked in different laboratories, though the results could not be universally confirmed.

In summary, hemangiomas express abnormal proliferation with increased endothelial cell turnover and increased surface markers (VEGF, bFGF) [3].

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Vascular Malformations

Conversely, vascular malformations are a product of abnormal morphogenesis. Their endothelial lining is flat, smooth muscle architecture is abnormal, and the expression of surface markers is minimal. Vascular malformations are made up of dysplastic vessels without cellular proliferation, and they have no propensity to regress.

Classification of Vascular Lesions

In the past, the terminology used in association with such abnormalities has been rather confusing, being mainly descriptive and dismissive of the histological origin of the lesions. A binary classification, into *vascular tumors* and *vascular malformations*, was proposed by Mulliken and Glowacki in 1982 [4] and has since been adopted by the International Society for the Study of Vascular Anomalies (ISSVA), established in 1992 (Table 50.1). As more disease entities have been identified and more genetic basis has been discovered, in 2015, the classification was revised and an updated version was issued [5]. The extended detailed version of the classification is available on the ISSVA web site (www.issva.org).

The updated classification is based on the new genetic and histologic information available since 1996 and provides a more clinically relevant and flexible option. It can be used by all medical and surgical specialties.

Table 50.1 International Society for the Study of Vascular Anomalies classification system [6]

Vascular (or vasoproliferative) neoplasms	Vascular malformations
Infantile hemangioma	Slow-flow vascular malformations
Congenital hemangiomas Rapidly involuting congenital hemangioma (RICH) Non-involuting congenital hemangioma (NICH)	Capillary malformation Venous malformation Lymphatic malformation
Kaposiform hemangioendothelioma and tufted angiomas (with or without Kasabach–Merritt syndrome)	Fast-flow vascular malformations
Spindle cell hemangioendothelioma	Arterial malformation
Epithelioid hemangioendothelioma	Arteriovenous malformation
Other rare hemangioendotheliomas (i.e., composite, retiform, and others)	Arteriovenous fistula
Angiosarcoma	Combined vascular malformations (various combinations of the above)
Dermatologic acquired vascular tumors (i.e., pyogenic granuloma)	–

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In order to understand different modalities of investigation and treatment, it is important to appreciate the different origin and behavior of vascular anomalies irrespective of their site.

We describe the main lesions with special emphasis on gastrointestinal (GI) lesions.

Group I: Vascular Tumors

Vascular tumors are divided into the following major groups [5]:

1. Benign:
   - Infantile hemangiomas
   - Congenital hemangiomas (CH):
     - Rapidly involuting congenital hemangiomas (RICH)
     - Non-involuting congenital hemangiomas (NICH)
     - Partially involuting CH (PICH)
   - Tufted angioma
   - Spindle cell hemangioma
   - Epithelioid hemangioma
   - Pyogenic granuloma (or lobular capillary hemangioma)
   - Others
2. Locally aggressive or borderline:
   - Kaposiform hemangioendotheliomas
   - Retiform hemangioendothelioma
   - Papillary intralymphatic angioendothelioma
   - Dabska tumor
   - Composite hemangioendothelioma
   - Kaposi sarcoma
   - Others
3. Malignant:
   - Angiosarcoma
   - Epithelioid hemangioendothelioma
   - Others

Infantile hemangioma is the most common tumor of infancy and childhood with recorded 1–2.6% incidence among Caucasian neonates. By the age of 1, some 4–12% of Caucasian children have hemangioma. The female-to-male ratio is 3:1. Hemangiomas are more common in premature babies [7]. They are usually apparent in the early neonatal period, proliferate until 1 year of age, and then slowly regress until the age of 7 when they end in the involution phase.

The hemangiomas of the GI tract only account for approximately 1% of all GI tumors [8]. They have a tendency toward multiplicity (intestinal hemangiomatosis), and solitary tumors are uncommon. They are often associated with cutaneous hemangiomas. If patients present with five or more cutaneous hemangiomas, the abdominal ultrasound

scan is indicated to look for visceral lesions. When present they are more commonly situated in the liver [9].

Overall, visceral vascular anomalies manifesting gastrointestinal symptoms are rare. Patients may present with GI hemorrhage, anemia, or symptoms of obstruction. An intestinal hemangioma rarely bleeds after the proliferative phase has ended. Therefore, occurrence of gastrointestinal bleeding secondary to hemangiomas after the first year or age 2 is very unlikely.

Eleven to thirty percent of GI hemangiomas may remain asymptomatic [10, 11].

Group II: Vascular Malformations

Although most vascular lesions are sporadic, inheritance has been observed and has thus provided a route to genetic analysis. Sporadic forms usually present as single lesions, but multiple lesions have been observed in familial cases.

Ninety percent of vascular malformations are present at birth. Estimated incidence of vascular anomalies in the general population is approximately 1.5% [12].

Female-to-male ratio is 1:1. These malformations infiltrate the surrounding tissue, never regress, and enlarge with the growth of the patient. Their growth can be worsened over time if not treated. Changes in hemodynamic factors such as arteriovenous shunting or venous stasis especially during puberty or pregnancy can accelerate growth and morbidity.

The "vascular malformations" group can also be subdivided into fast flow (arterial or arteriovenous) and slow flow (capillary, lymphatic, or venous). If fast-flow lesions are localized subcutaneously, they may become more erythematous and may develop a palpable thrill and a bruit.

Vascular malformations of the GI tract are rare in children, but they can be diagnosed at any age, including newborns [13]. They may appear anywhere in the GI tract from the mouth to the anus and may coexist with similar lesions on the skin.

Patients may present with recurrent abdominal pain; GI bleeding; acute or, more often, chronic anemia; intestinal obstruction; volvulus; intussusception; and palpable mass lesion. Sometimes these lesions might be mistaken for inflammatory bowel disease due to mucosal edema, nodularity, and vascular congestion. Bleeding per rectum may also raise differential diagnosis of symptomatic Meckel's diverticulum. Intestinal lesions often represent a diagnostic challenge requiring sophisticated methods of investigation.

Associated Syndromes

Certain syndromes are associated with GI vascular malformations, including:

1. Osler–Weber–Rendu disease (hereditary hemorrhagic telangiectasia)
2. Klippel–Trenaunay syndrome
3. Blue rubber bleb nevus (BRBN) syndrome
4. Proteus syndrome

1. Osler–Weber–Rendu Disease (Hereditary Hemorrhagic Telangiectasia)

This is a genetic disorder inherited in autosomal dominant manner occurring in 1:5000 of the general population. Genetic diagnosis is difficult, but tests are available for the *ENG*, *ACVRL1*, and *MADH4* mutations [14].

The condition typically presents with distinctive small skin and mucosal vascular malformations (telangiectasias) and may appear in the nose, lips, fingers, and along the GI tract. Larger arteriovenous malformations are usually localized in the lungs, liver, brain, and, occasionally, the spine. Though the main clinical problem is usually epistaxis, GI bleeding may also occur, but it is rare in pediatric population. The malformations developing in the liver may eventually lead to portal hypertension, high-output cardiac failure, or encephalopathy. The clinical diagnosis is based on the Curaçao criteria [15], which are:

- Spontaneous recurrent epistaxis
- Multiple telangiectasia in typical locations
- Proven visceral arteriovenous malformations (lung, liver, brain, spine)
- First-degree family member with hereditary hemorrhagic telangiectasia

Management Usually symptomatic treatment of anemia due to GI bleeding is the only required treatment, but in severe cases, argon plasma coagulation or laser therapy may be applied.

2. Klippel–Trenaunay Syndrome

This is a relatively rare condition with approximate incidence of 1:30,000 live births [16]. Historically, it is characterized by a triad of symptoms: capillary skin malformation (port wine stain), varicose veins (especially persistent embryonic lateral vein of Servelle), and bony/soft tissue hypertrophy of the extremity. This hypertrophy is caused by venous and lymphatic malformations localized in the soft tissue but often involving the bony structures. Most commonly, one extremity is affected, usually the leg, but multiple limb involvement is also possible.

The etiology of the syndrome is not entirely clear, but it is believed that most cases happen due to sporadic gene mutation, though some autosomal dominant inheritance cases are

also reported. Mutations in PI3K-AKT gene pathway may play a role in the development of the condition [17].

In association with the classical triad of clinical symptoms, the GI tract may also be affected by vascular malformations. Gastrointestinal tract involvement is observed in up to 20% of cases, usually in the rectum and sigmoid, which can manifest as gastrointestinal bleeding, usually during the first decade of life.

Management Angiographic intervention such as embolization of the bleeding vessel can sometimes be successful. Endoscopic therapy is usually preferred for localized lesions or postoperative residual disease. Endorectal resection extending to the transverse colon has been reported in cases of more extensive disease [18].

3. BRBN Syndrome (Bean Syndrome)

This is a very rare syndrome with slightly over 200 case reports in the literature. It is characterized by cutaneous and visceral venous malformations. These malformations can occur in any tissue but are most prominent in the skin and in the GI tract, the small bowel being the most frequently affected part. Their number can range between several to hundreds. In 2005, Fisherman et al. reported 557 lesions in one patient [19]. Cutaneous lesions are generally small (about 1–2 cm or even smaller), rubbery, compressible, blue to purple nipple-like nodules often found on the face, the upper limbs, and the soles of the feet.

Most cases are sporadic, but some autosomal dominant transmission has also been reported.

A common problem is GI bleeding presenting at early age and continuing throughout life, resulting in chronic anemia requiring repeated blood transfusions. Most patients respond to conservative therapy, which includes iron supplementation, octreotide, and blood transfusions. However, steroids, propranolol, interferon α2a, and sirolimus are sometimes used to decrease angiogenesis. Severe cases may require surgical excision [20]. Sirolimus has been reported to be a successful treatment for BRB [21].

Management A broad spectrum of treatment options is available, from conservative and endoscopic treatment to an aggressive surgical approach. Recently, various endoscopic treatments for gastrointestinal lesions have been suggested including injection of sclerosants [22]. The simple and relatively non-invasive nature of polidocanol injection suggests that it may be the treatment of choice for pediatric and adolescent patients with BRBNS.

4. Proteus Syndrome

This syndrome associates multiple nevus flammeus, body hemihypertrophy, partial achral gigantism, intra-abdominal lipomatosis, pachyderma involving the palmar and plantar aspects of the hand and foot, hypertrophy of vertebral bodies, venous and lymphatic malformations, and epidermal nevi [23].

Imaging of gastrointestinal venous malformations includes plain film. Phleboliths are characteristic of venous malformation and can occasionally be seen on plain film.

Lymphatic Malformations

Lymphatic malformations are relatively rare in the GI tract, especially in children.

The majority of intra-abdominal lymphatic malformations are identified within the mesentery and retroperitoneum. Lesions affecting the bowel are uncommon and account for less than 1% of all lymphatic malformations. Approximately, 70% of those are detected in the small bowel and 30% in the colon [24].

Patients may present with abdominal pain, failure to thrive, palpable cystic mass, intestinal volvulus, bowel obstruction, or intussusception. Differential diagnosis includes intestinal duplication cyst, ovarian cyst, teratoma, Wilms' tumor, and neuroblastoma. In the case of a small bowel lesion, the differential diagnosis of lymphangiomyoma and benign multicystic mesothelioma could be considered. Immunohistochemical analysis can give a clear indication if the lesion was derived from the lymphatic vessels (expected focal positivity of D2–40, a lymphatic endothelial marker).

Management Surgical treatment with complete resection is recommended in all cases due to the high risk of complications related to the increase in the size of the lesion. Sclerotherapy with a variety of sclerosing agents has been reported as a less invasive treatment option, but it has a variable recurrence rate with up to 100% in some series [25].

Primary Intestinal Lymphangiectasia (Waldmann's Disease)

Dilated intestinal channels resulting in lymph leakage into the small bowel lumen and responsible for protein-losing enteropathy leading to lymphopenia, hypoalbuminemia, and hypogammaglobulinemia characterize this disorder. The exact etiology of this condition is unknown, and it is generally diagnosed before 3 years of age. Patients often present with bilateral lower limb lymphoedema, inability to gain weight, and steatorrhea and later develop pleural effusions or ascites. This may be accompanied by abdominal pain. Sometimes the abdominal mass may be palpable, or patients may develop intussusception. Mechanical obstruction of the small intestine may be caused by localized edema leading to intestinal wall thickening and lumen diminution.

Management Diagnostic process can be helped by ultrasound scan, capsule endoscopy, CT, albumin scintigraphy, and sometimes lymphoscintigraphy.

The condition requires long-term medical treatment in the form of reduced dietary fat, medium-chain triglycerides, elemental diet, corticosteroids, and vitamin supplements [26]. In some localized cases, surgical resection may provide satisfactory results [27]. In chronic recurrent conditions, fibrin glue application has been used.

Investigations

The diagnosis of a visceral vascular lesion is often suggested by the patient's age, clinical presentation, and the appearance of associated cutaneous lesions. When investigating a possible GI lesion, aim should be employed toward the following:

- Diagnosis of the main lesion: type, location, relation to the other organs, etc.
- Diagnosis of associated anomalies: syndromes
- Hematological status
- Modalities of imaging

Several imaging modalities can be employed to establish more precisely the type and the location of vascular anomalies. MRI imaging, selective angiography, color Doppler sonography, multiphase CT enterography, and 99mTc-red blood cell (RBC) scintigraphy can all be useful for this purpose [28–31]. The slow-flow veno-lymphatic malformations on MRI scan are typically multispatial and multicystic and may have a partially solid component. Fast-flow arteriovenous malformations demonstrate turbulence, flow voids, and hyperintense signal.

From the imaging point of view, it is important to remember that while young the hemangiomas may behave like arteriovenous malformations and may exhibit the features of fast flow. The slow flow is being established in involution phase.

Currently, ultrasonography and MRI with contrast (T1- and T2-weighed sequences) are the most widely used modalities of initial diagnostic choice. MRI is more sensitive than CT in detecting venous malformations and can show more information regarding bowel wall thickening, fat infiltration, and vessels appearance or identify the presence of any soft tissue masses.

Nuclear scintigraphy can be useful to detect a venous malformation or to demonstrate a bleeding site.

At endoscopy, the whole GI tract should be visualized, if possible, by using different approaches, including rigid or flexible endoscopy, single- or double-balloon enteroscopy, and wireless capsule endoscopy. Each method has its advantages and limitations. Capsule endoscopy, for example, transmits a radio-frequency signal, which allows estimation of the location of the capsule and tracking of it in real time inside the GI tract. Nevertheless, unlike endoscopy it cannot treat pathologies it discovers. Another limitation, specific to the pediatric population, can be the age and size of the patient; these factors can affect tolerance of certain procedures and the accuracy of imaging.

Sometimes, the diagnosis is made during surgery which can be either laparotomy or laparoscopy and enterotomies.

Biopsies should be avoided because of the high risk of bleeding.

Treatment

Even though both hemangiomas and vascular lesions often present with bleeding, principal treatment options are entirely different.

Vascular Tumors

Visceral hemangiomas are at risk of bleeding in the proliferation phase, and the risk diminishes after they enter the involuting phase. In many cases, this could be achieved by using the inhibitors of angiogenesis. For example, extensive intestinal hemangiomatosis can be successfully managed with propranolol, corticosteroids, α-interferon, vincristine, and also thalidomide and somatostatin analog [32].

Vascular Malformations

Sclerotherapy

If amenable, especially if the vascular malformations are located in the anorectal region, direct injection and sclerotherapy can be performed [33].

The principal method of sclerotherapy is injection of water-based or oleous substances into abnormally dilated vessels that can inflict damage to endothelial lining and cause thrombosis, fibrosis, stenosis, and—eventually—scarring. Sclerotherapy for treatment of lymphatic and venous malformations employs a variety of agents, including absolute ethanol, bleomycin, OK-432 (picibanil), and doxycycline [2]. Each agent has a risk profile: Ethanol injections are painful, requiring general anesthesia even in adults, and may cause cardiovascular shock. Bleomycin may cause interstitial pneumonia or pulmonary fibrosis, and treatment with doxycycline may result in tooth discoloration or electrolyte abnormalities.

Medical Therapy

The antiangiogenic treatment is ineffective for vascular malformations. Some complicated vascular anomalies resistant to other treatments can be conservatively treated with sirolimus (especially if they have a lymphatic component), bevacizumab, and estrogen–progesterone combination therapy [15, 34].

Surgical Therapy

Once diagnosed, treatment options are segmental or wedge bowel resection band or suture ligation and argon plasma coagulation or electrocoagulation [35].

Conclusion

GI vascular lesions are rare. Vascular malformations are more common than vascular neoplasms. They require extensive investigations to identify the type and location. Different modalities need to be used to treat the lesions by limited resection or sclerotherapy. Many lesions require lifelong supportive therapy to treat chronic anemia and nutritional deficiencies.

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Polyps and Other Tumors of the Gastrointestinal Tract

51

Warren Hyer, Marta Tavares, and Mike Thomson

Introduction

Children and adolescents with gastrointestinal polyps may present symptomatic with rectal bleeding, abdominal pain, or intussusception or alternatively, they may be asymptomatic, having been referred, either because an adult family member has been affected with early-onset colorectal cancer (CRC) or there is a known family history of a polyposis syndrome. Many children fall under the latter category. Managing these children and families requires knowledge of the different polyposis syndromes, their inheritance and genetics, potential for malignant change, and pediatric complications.

Gastrointestinal polyps in children fall into two major categories: hamartomas and adenomas (Table 51.1). Solitary polyps in children are most commonly hamartomas, predominant of the juvenile type, and such polyps are benign. Of the familial syndromes, familial adenomatous polyposis (FAP) is more common than juvenile polyposis or Peutz–Jeghers polyposis.

Table 51.1 Polyps and polyposis syndromes seen in childhood

Adenomatous polyposis syndromes
Familial adenomatous polyposis (FAP)
MYH-associated polyposis (MAP)
Turcot syndrome
Lynch syndrome
Hamartomatous polyps
Solitary juvenile polyp
Juvenile polyposis syndrome
PTEN hamartoma tumor syndrome
Peutz–Jeghers syndrome
Inflammatory polyps

PTEN phosphatase and tensin homologue deletion, *MYH* mutY human homologue

Clinical Presentation of Gastrointestinal Polyps

The most common clinical manifestation of a large bowel polyp is painless rectal bleeding. Other symptoms attributed to polyps include abdominal pain, altered bowel habit, and prolapse of polyp or rectum. Diagnosis will be made after full colonoscopy with polypectomy. The clinical presentation, endoscopic appearance, pathological findings, histological description of the polyp, and genetic investigations are all necessary to establish the correct diagnosis of a polyposis syndrome. Once a polyp has been identified at endoscopy, a carefully targeted family history must be taken to enquire if there are family members who have or have had cancer, the site of the cancers, and age of onset (see Table 51.2). Taking such a history to develop a detailed family cancer pedigree may require the expertise of a multidisciplinary familial cancer clinic or polyposis registry [1].

The Single Hamartomatous Polyp: The Juvenile Polyp

With a prevalence of up to 3% of children, the most common presentation of a single hamartomatous (or juvenile) polyp is painless bright red rectal bleeding with blood seen on wiping or mixed in the stool. Seventy percent of juvenile polyps are found in the rectum or rectosigmoid. The rest however are found more proximally, hence the need for pancolonoscopy. Single or solitary juvenile polyps are benign and confer no future risk of malignancy. Presently, it is unknown whether

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Table 51.2 History and examination in a child with possible GI polyps

History
Nature of bleeding and frequency
Painful or painless rectal bleeding
History of GI obstructive symptoms
Detailed family history exploring early deaths or diagnosis of GI cancer and include history of non-GI malignancies
Weight loss, anorexia (tumor)
Learning difficulties (JPS or PTEN hamartoma)
Examination
Mucosal pigmentation (PJS)
Dysmorphic features (JPS)
Edema (hypoalbuminemia in infantile JPS)
Extra intestinal manifestations of FAP
Hepatic mass (FAP)
Cutaneous telangiectasia (HHT with JPS)
Thyroid mass (FAP or Cowden)

JPS juvenile polyposis syndrome, *PTEN* phosphatase and tensin, *PJS* Peutz–Jeghers syndrome, *FAP* familial adenomatous polyposis, *HHT* hereditary hemorrhagic telangiectasia

children who present with a single polyp in childhood may continue to form polyps over time [2].

If a polyp is found to be solitary after full colonoscopy, and there is no relevant family history, endoscopic polypectomy should be sufficient treatment. After polypectomy, parents should be advised that juvenile polyps may be the first feature of a hamartomatous polyposis syndrome, and if new symptoms arise, the child should be reinvestigated. If multiple juvenile polyps are found (>3–5) or there is a positive family history (e.g., colonic polyps or early-onset CRC), a hamartomatous polyposis syndrome should be considered, and an alternative approach should be taken.

Hamartomatous Polyposis Syndromes

Hamartomatous polyposis syndromes are a rare group of hereditary autosomal dominant disorders. The polyps themselves are benign, but the polyposis syndromes confer a significant potential for developing CRC and extra colonic cancers. The hamartomatous polyposis syndromes include juvenile polyposis syndrome (JPS), Peutz–Jeghers syndrome (PJS), and phosphatase and tensin (PTEN) hamartoma tumor syndrome, which includes Cowden (CS) and Bannayan–Riley–Ruvalcaba syndrome (BRRS).

Juvenile Polyposis Syndrome

JPS is rare, with an estimated prevalence of 1 in 100,000 individuals presenting with multiple hamartomatous polyps and an increased risk of gastrointestinal malignancies. Affected individuals develop multiple gastrointestinal polyps which are predominantly in the colon, though other areas of the gastrointestinal tract can also be involved. The condition should be considered in any patient with more than five juvenile polyps in the colon, any juvenile polyps found in other parts of the gastrointestinal tract, or if any juvenile polyp is found in a child with a positive family history. Patients present with chronic and acute gastrointestinal bleeding, anemia, prolapsed rectal polyps, abdominal pain, and diarrhea and can develop up to 50–200 polyps in the colon, while in some patients, with generalized juvenile polyposis, polyps can affect the colon, stomach, and small bowel. The total number of polyps needed to make the diagnosis remains controversial, between 3 and 5 [3]. A significant proportion of patients with juvenile polyposis have been reported to have other morphological abnormalities including digital clubbing, polydactyly, macrocephaly, alopecia, cleft lip or palate, congenital heart disease, and mental retardation. A specific variant of JPS called juvenile polyposis of infancy has an onset in infancy presenting with anemia and hemorrhage, diarrhea, protein-losing enteropathy, intussusception, and rectal bleeding. The course in such infants is fulminant, and, even despite colectomy, in severe cases, death occurs before the age of 2 years.

There is little doubt that juvenile polyposis is a premalignant condition. There is a 15% incidence of colorectal carcinoma occurring in patients under the age of 35 years, leading to a cumulative lifetime risk of CRC of 38–68% with a mean age of colonic neoplasia onset between 38 and 44 years [4].

Genetics of Juvenile Polyposis

JPS is a fully penetrant condition with variable expression. Sixty percent of cases are familial and the others occur sporadically. Germline mutations in *SMAD4*, *BMPR1A*, and *ENG1* cause JPS. Approximately 54% of JPS cases will have a detectable mutation [5].

Patients with the *SMAD4* mutation on chromosome 18q21.2 appear to have a higher risk of gastric polyps and hereditary hemorrhagic telangiectasia (HHT). The latter condition is characterized by cutaneous telangiectasia and risk of arteriovenous malformations. Patients found to have the *SMAD4* mutation should be screened for cerebral and pulmonary arteriovenous malformations associated with HHT [6]. *BMPR1A* is located on chromosome 10q22.3 and accounts for another 20% of JPS patients. *ENG1* mutation on gene 9q34.1 has recently been described in JPS patients without HHT.

Screening and Follow-Up

Once the gene mutation has been identified in the index patient, other at-risk family members should be tested. All children of an affected adult will have a 50% chance of inheriting the mutation, and if the family mutation is known, children should undergo genetic screening.

Current guidelines suggest affected children should undergo colonoscopic surveillance every 3 years or earlier if symptoms arise [7]. In those families where the gene mutation is not known, first-degree relatives of patients with JPS should be screened by a single colonoscopy starting at age 12–15 years, even when the subject is asymptomatic (Fig. 51.1).

Full colonoscopy is necessary as right-sided polyps are more common. All polyps should be resected. Annual colonoscopy is performed until all polyps have been resected after which the screening interval is stretched to every 2–3 years. Gastroscopy is commenced from mid-teens. Colectomy is warranted for patients with cancer, dysplasia, or high polyp burden with symptoms that cannot be controlled endoscopically.

PTEN Hamartoma Tumor Syndrome

This group comprises three rare genetic syndromes: Cowden syndrome, BRRS syndrome, and Proteus syndrome (the latter has very few intestinal features). All are associated with a mutation in the PTEN gene located at 10q23.3 [8]. All three syndromes are characterized more by extraintestinal manifestations than intestinal polyposis. The PTEN mutation can be detected in 80% of patients with Cowden, 60% with BRRS, and 50% with Proteus syndrome.

Cowden syndrome rarely presents in childhood. Clinical manifestations include macrocephaly, papillomatous papules, mucocutaneous lesions, facial trichilemmoma, and acral keratosis. It carries a 50% risk of breast cancer in adult women and a 10% lifetime risk of epithelial thyroid cancer. Guidelines presently recommend screening for breast, thyroid, endometrial, and kidney cancer from the age of 18–25 years.

BRRS presents in childhood with gastrointestinal hamartomas, particularly in the ileum and colon, which cause intussusception, rectal bleeding, and hypoalbuminemia. There are additional characteristics including macrocephaly,

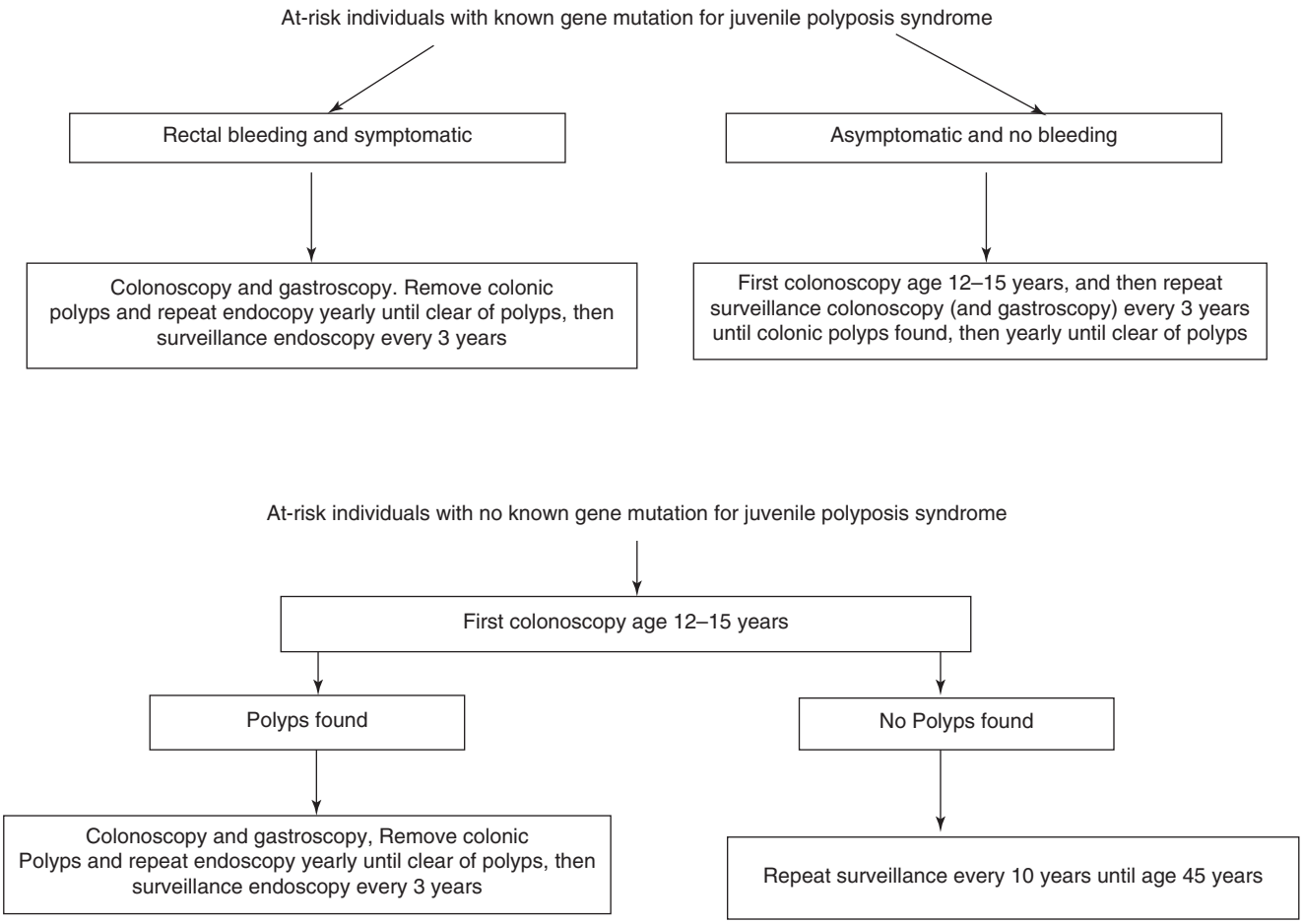

Fig. 51.1 Screening and surveillance algorithm for juvenile polyposis syndrome

developmental delay, abnormal metacarpal and phalanges, pectus excavatum, scoliosis, genital pigmentation, and hemangiomatosis. BRRS presents prior to adolescence, and there is value in genetic testing in early childhood in a family where the mutation has been identified. Patients with BRRS need regular colonoscopy and small bowel surveillance as they are at risk of anemia, intussusception, and hypoalbuminemia from the polyposis. They carry a probable lifetime increased risk of cancer, and surveillance is recommended from age 18–25 years focusing on renal, thyroid, and breast cancer [9].

Peutz–Jeghers Syndrome

Clinical Features and Diagnosis

PJS is a rare autosomal dominant condition with a prevalence of 1 in 50,000 to 1 in 200,000 live births. It is characterized by mucocutaneous pigmentation and the presence of hamartomatous polyps throughout the gastrointestinal tract. Polyps arise primarily in the small bowel and to a lesser extent in the stomach and colon. Polyps are most commonly found in the jejunum and cause bleeding and anemia or intussusception and obstruction from an early age. Presumptive diagnosis can be made in those with a positive family history and typical PJS freckling.

Pigmentation tends to arise in infancy, occurring around the mouth, nostrils, perianal area, fingers, toes, and the dorsal and volar aspects of hands and feet. The primary concern to the pediatrician is the risk of small bowel intussusception causing intestinal obstruction, vomiting, and pain (Fig. 51.2).

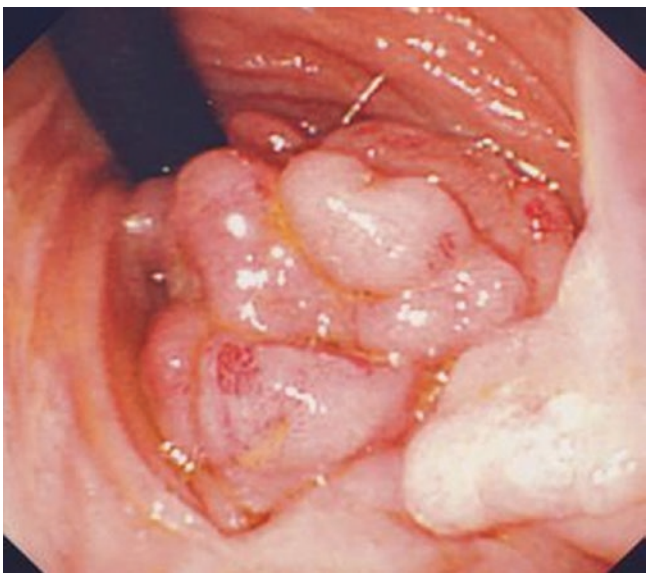

Fig. 51.2 Large obstructing duodenal PJS polyp seen at gastroscopy

In addition, intestinal bleeding—with melena, hematemesis, and rectal bleeding—can occur, leading to anemia.

Genetics of PJS

As with other hamartomatous syndromes, PJS has an autosomal dominant pattern of inheritance, and many cases may be sporadic new mutations. The mutated gene *STK11(LKB1)*, located on chromosome 19p 13.3, can be identified in up to 90% of PJS patients [10]. After appropriate genetic counseling and informed consent, testing at-risk family members may be performed early in childhood so that gastrointestinal surveillance can commence before gastrointestinal complications arise.

Screening, Management, and Complications

Individuals at risk of PJS should be evaluated in infancy for pigmented lesions and gastrointestinal symptoms. Asymptomatic at-risk children should undergo genetic testing for the family proband mutation in the STK11/LKB1 mutation if known, soon after infancy, so the family can access medical care early if the child develops symptoms consistent with small bowel obstruction.

The management of a young child with mid-gut PJS polyps is controversial, but recommended guidelines have been published [11]. Children who present with mid-gut intussusception need urgent polypectomy either by laparotomy or laparoscopy and intraoperative enteroscopy (IOE, Fig. 51.3). An IOE is recommended in any patient with PJS undergoing laparotomy, as careful endoscopy via an enterotomy in the small bowel allows identification and removal of polyps, thus avoiding multiple enterotomies and the risk of short bowel syndrome associated with

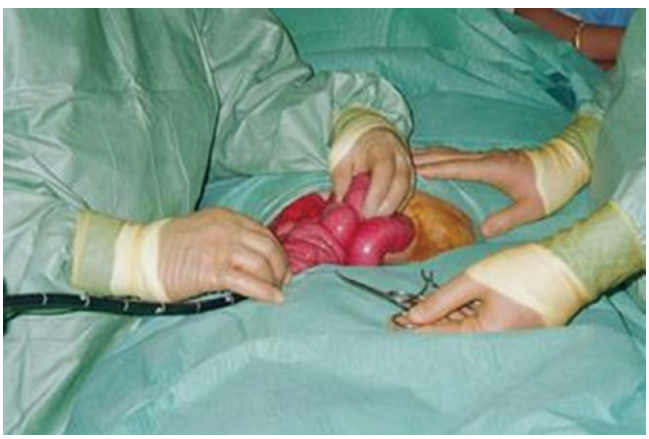

Fig. 51.3 Intraoperative enteroscopy performed at laparotomy for intussusceptions in a patient with PJS

resection. This technique is superior to palpation and transillumination in identifying polyps, and removal of all detected polyps ("clean sweep") reduces re-laparotomy rate significantly [12].

Endoscopic evaluation of the upper and lower gastrointestinal tract and imaging of the small bowel should be performed from the age of 8 years. Screening should start earlier if symptoms are present before this. Video capsule endoscopy (VCE) has replaced barium enterography as the preferred technique for assessing the small bowel [13]. VCE is more sensitive, preferred by patients, and reduces the lifetime risk from cumulative radiation exposure. An acceptable alternative to VCE is MRI enterography with a close correlation between the two modalities, especially with polyps >15 mm [14]. The advantages and disadvantages of prophylactic polypectomy for asymptomatic patients should be discussed with the family (Fig. 51.5).

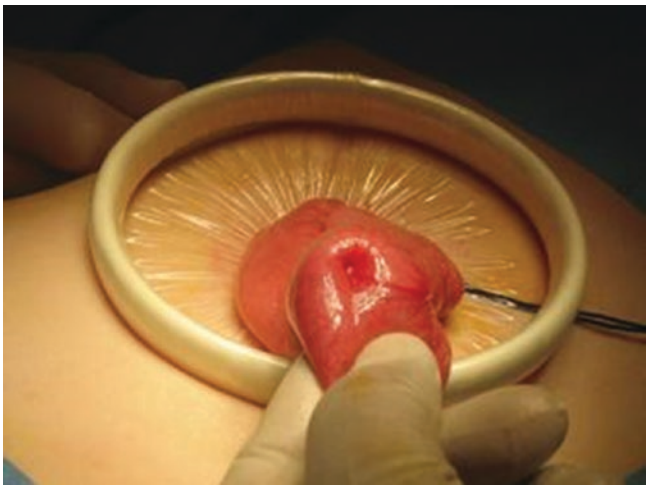

Fig. 51.4 Indentation of the serosal surface of the ileum from intussuscepting PJS polyp

Prophylactic polypectomy of larger small bowel polyps (>1.5 cm) by laparoscopy or double-balloon enteroscopy (DBE) should be performed in order to reduce the incidence of subsequent complications and the requirement for emergency laparotomy. Polypectomy via DBE in the small bowel carries a significant risk of perforation and should be performed only by those who are expert in polypectomy. Muscularis mucosa commonly invaginates into the large pedunculated stalk increasing the risk of perforation at electrocautery (Fig. 51.4). DBE can be combined with laparoscopy to assess perforations that may arise at polypectomy.

The risk of neoplasia is well documented in young adults. A meta-analysis to assess the risk of cancer in PJS identified a relative risk for all cancers in PJS patients (aged 15–64) of 15.2 compared to the normal population, with tumors. Clinicians caring for adolescents with PJS should be aware of unusual symptoms and have a low threshold for investigating potential malignancies. A recommended screening program for PJS patients after adolescence is shown in Table 51.3.

Table 51.3 Suggested program for screening for malignancies in Peutz–Jeghers syndrome after adolescence

General
Annual hemoglobin and liver function tests
Annual clinical examination
Genital tract
Annual examination and testicular US biennial from birth until 12 years
Cervical smear every 3 years from age 25 years
Breast
Monthly self-examination from age 18 years
Annual breast MRI from age 25 to 50, thereafter annual mammography

EGD esophagogastroduodenoscopy, *VCE* video capsule endoscopy, *MRI* magnetic resonance imaging

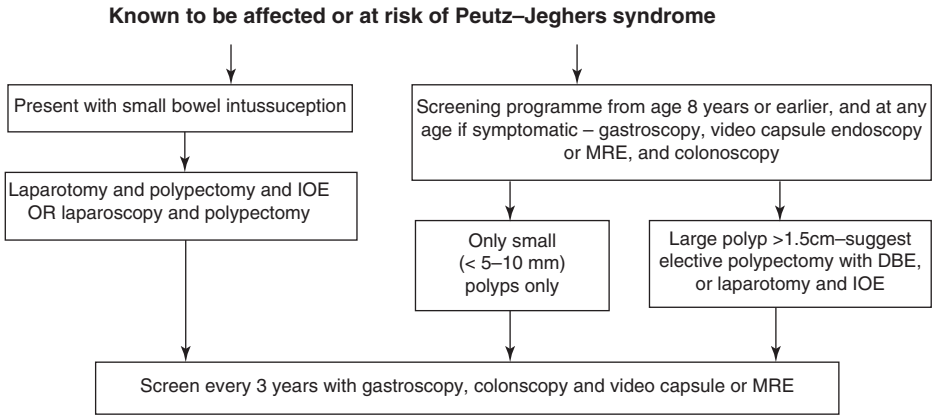

Fig. 51.5 Screening and surveillance algorithm for Peutz–Jeghers syndrome. MRE magnetic resonance enterography, DBE double-balloon enteroscopy, IOE intraoperative enteroscopy

Adenomatous Polyposis Syndromes

Familial Adenomatous Polyposis

In children, GI adenomas are almost always associated with hereditary adenomatous polyposis syndromes. FAP is characterized by the development of hundreds of adenomas in the colon and rectum as well as several extra colonic manifestations. Almost all affected patients will develop a CRC if not detected and treated at an early stage.

Clinical Features

Patients with FAP typically develop multiple adenomas throughout the large bowel—usually more than 100 and sometimes more than 1000 (Fig. 51.6). Polyps begin to appear in childhood or adolescence and increase in number with age. The standard clinical diagnosis of typical/classical FAP is based on the identification of >100 colorectal adenomatous polyps. By the fifth decade, CRC is almost inevitable if colectomy is not performed. Attenuated FAP (AFAP) is a milder form of the disease which is observed in 8% of cases. It is characterized by fewer adenomas and later presentation [15].

Gastric fundic gland polyps occur in the antrum in 50% of FAP-affected adults, and small bowel adenomas also occur and neither requires intervention. Children under 5 years of age may develop hepatoblastoma, with an increased risk in boys [16]. Adult patients are at increased risk of malignancies of the duodenum and ampulla of Vater, with a lifetime risk of duodenal adenomas of 100%. Duodenal adenomas will progress to malignancy if untreated in 5% of adults and are the second most common malignancy in FAP and AFAP. In addition, FAP is associated with an increased risk of cancers of the thyroid, brain, and pancreas, while papillary carcinoma of the thyroid has been reported in adolescence.

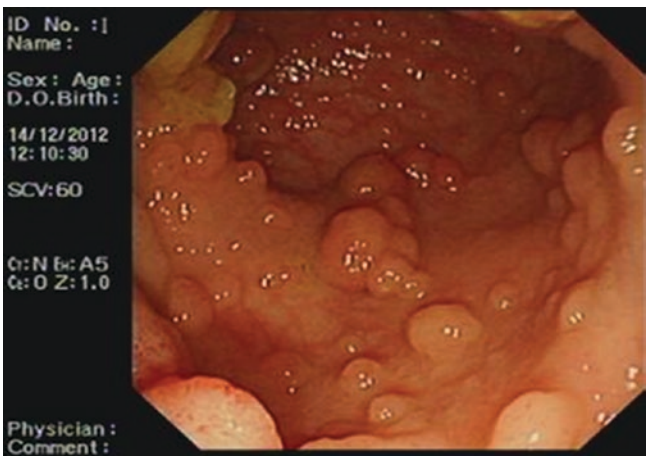

Fig. 51.6 Endoscopic appearance of large adenomatous polyps seen in advanced FAP

Table 51.4 Extracolonic manifestations of FAP in children and young adults

Site	Examples
Bone	Osteomas, mandibular, and maxillary
	Exostosis
	Sclerosis
Dental abnormalities	Impacted or supernumerary teeth
	Unerupted teeth
Connective tissue	Desmoid tumors
	Excessive intra-abdominal adhesions
	Fibroma
	Subcutaneous cysts
Eyes	Congenital hypertrophy of the retinal pigment epithelium
CNS	Glioblastomas, for example, Turcot syndrome
Adenomas	Stomach
	Duodenum
	Small intestine
	Adrenal cortex
	Thyroid gland
Carcinomas	Thyroid gland
	Adrenal gland
Liver	Hepatoblastoma

Extraintestinal manifestations are common (see Table 51.4). Pigmented ocular lesions (previously termed congenital hypertrophy of the retinal epithelium or CHRPE) are found in some but not all cases.

Genetics of FAP

FAP is an autosomal dominant inherited condition caused by a mutation in the adenomatous polyposis coli (APC) gene occurring in 1:10,000 births. In 20–30% of cases, the condition is caused by a spontaneous mutation with no clinical or genetic evidence of FAP in the parents or family.

The gene responsible for FAP, *APC*, is located on chromosome 5q21 and appears to be a tumor suppressor gene [17]. Most mutations are small deletions or insertions which result in the production of a truncated APC protein which then predisposes to adenoma formation. Many mutations have been identified on this large gene, and there is a correlation between the genetic site and severity of clinical manifestation. Mutations between codons 1250 and 1464 (Fig. 51.7), and especially those with a mutation at codon 1309, are associated with a severe form of FAP. Mutations localized at the extreme ends of the gene and in the alternatively spliced part of exon 9 are associated with a mild form of FAP, and an intermediate expression of disease is found in patients with mutations in the remaining parts of the gene. Other phenotype–genotype correlations have been observed [18]. These correlations are not absolute, and there may be considerable intrafamilial variation suggesting that there are other factors involved

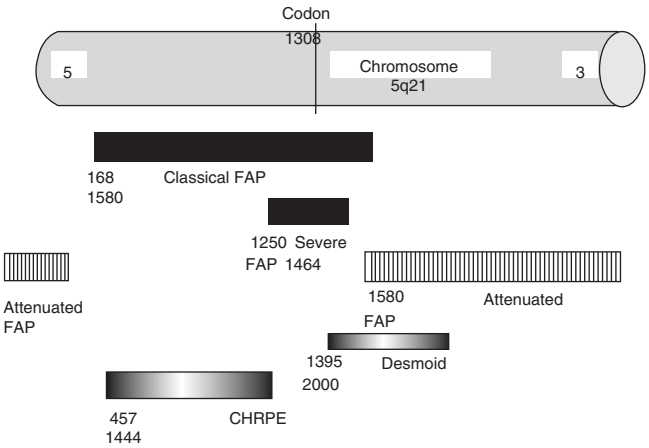

Fig. 51.7 APC protein domains showing FAP genotype–phenotype correlation with codon number. APC adenomatous polyposis coli, FAP familial adenomatous polyposis, CHRPE congenital hypertrophy of the retinal epithelium

in the pathogenesis of the disease. Families need to be aware that the mutation may only be detected in 70–90% of cases.

Diagnosis: Interpretation of the Genetic Test and Clinical Screening in FAP

In order to determine the appropriate screening protocol for a given family, the first step would be to seek which mutation is present in the FAP affected index case. If a mutation cannot be found, the genetic testing is non-informative, and it will not be possible to offer predictive testing to asymptomatic at-risk relatives.

When family-specific mutation is identified, directed DNA diagnostic techniques can be readily used to predict FAP in other family members. The absence of the gene mutation in other family members is considered accurate in excluding FAP, and the subject should be considered to hold an average population risk for the subsequent development of adenomas and cancer. Such genotype negative individuals can be discharged from follow-up.

The presence of the family-specific gene mutation confirms the diagnosis of FAP, and such patients should undergo endoscopic assessment. The diagnosis is confirmed by finding adenomas at colonoscopy (see Fig. 51.2). Teenagers predicted at gene testing to be affected will require a colonoscopy by the age of 14–16, preferably beginning at age 12–14 years [19], to determine polyp density and location, and degree of dysplasia. Upper endoscopic surveillance of the stomach, duodenum, and periampullary region with a side-viewing endoscope can be delayed until after the age 20 years, unless the patient has symptoms such as upper abdominal pain which warrant earlier investigation. In children from families in which the mutation is not known or cannot be identified, the genetic testing is non-informative, and it will not be possible to offer predictive testing to asymptomatic at-risk children. Protocols vary, but current approach is to perform colonoscopy on all first degree relatives from the age of 12–14 years every 3–5 years until adenomas are found. If by the age of 20 years, no adenomas have been identified despite the use of chromoendoscopy, colonoscopy should be performed at five yearly intervals.

No patient should undergo screening for FAP without detailed counseling. The individual being screened must understand the nature of the test and its possible outcomes. Many authorities feel that the child should be involved in the decision-making process and the diagnosis be delayed until the child is old enough to contribute to the screening program, for example, from the age of 12 years onward [20]. Although parents might request testing of a child or infant at a young age, there is value in deferring a test until the child or adolescent can participate in the discussion [21]. Well-informed consent, as a mature minor, prior to predictive testing is the most desirable outcome. There are psychological issues, as well as family, insurance, and employment implications, which may arise in the case of a positive result. These should be discussed prior to testing, and there should be a clear protocol for posttest management [22].

The current advice is to commence endoscopic surveillance from the early teens. Some patients, especially those with a mutation located at codon 1309 in the APC gene, may develop severe polyposis of the colorectum before the age of 10, and therefore, attention must be paid to FAP-related symptoms. These symptoms may include increasing bowel movements, looser stools, mucous discharge, rectal bleeding, abdominal, or back pain. In symptomatic patients, endoscopic investigation may be indicated at any age. Severe dysplasia and even malignancy have been documented in children with FAP under the age of 12 years. Consequently, those children from families in which severe dysplasia or carcinomas have been found at a young age should undergo screening at an earlier age [23]. Some clinicians advocate annual screening for hepatoblastoma with liver palpation, ultrasound, and serum á-fetoprotein levels in at-risk children between the ages of 0 and 6 years although current guidelines suggest this is not required [19].

Management of FAP

Internationally agreed guidelines are currently in place for the management of patients with FAP [24]. The role of colonoscopic surveillance is to assess adenoma burden and determine adenoma distribution especially in the rectum as these impact on surgical options for colectomy. Extra time should be spent in the rectum counting adenomas, especially those

= or >2 mm. If polyps are small or hard to visualize, chromoendoscopy should be considered to improve visibility of polyps.

There is no evidence for accelerated carcinogenesis and therefore no indication that the colonoscopy should be performed every year. The risk of developing cancer in teenage years is as low 0.2% so waiting 1–3 years between colonoscopies would appear safe, so long as families are not lost to follow up [25]. It should be acknowledged there is a phenotypic variation in this age group and the interval between colonoscopies needs to reflect this. Intrafamilial variation is well recognized so relying on family history alone is unsafe. The presence of a gene mutation associated with a more aggressive phenotype (e.g., codon 1309) should not dictate alone the timing of colonoscopy. The presence of symptoms, in particular rectal bleeding and/or anemia, suggests a significant polyp burden and requires an earlier colonoscopy.

Colectomy is currently the only effective therapy that eliminates the inevitable risk of CRC. In the absence of severe dysplasia, colectomy is usually performed in mid- to late teens or early 20s to accommodate work and school schedules. Almost all screen-detected adolescents are asymptomatic and therefore may not be willing to contemplate interruptions in their schooling or effects on relationships. The surgical option, therefore, must not only be carefully timed but also have low morbidity and excellent functional result.

There is no clear evidence to dictate at what polyp size or colonic burden should lead to colectomy and any recommendations remain arbitrary. Current practice is arbitrary recommending colectomy when there are many adenomas >10 mm, >500 of polyps >2 mm, or carpeting of the colon of polyps. The timing of primary preventative surgery may be influenced by knowledge of the mutation site and the likely severity of the polyposis. For example, patients with a deletion at codon 1309 should be offered earlier surgery since this phenotype is characterized by a large number of polyps and a higher risk of cancer [26].

Surgical options are either subtotal colectomy with ileorectal anastomosis (IRA) or restorative proctocolectomy with ileal pouch anal anastomosis (IPAA or pouch procedure). The IRA is a low-risk operation with good functional results and can be performed laparoscopically. There is no pelvic dissection, and therefore, attendant risks of hemorrhage, loss of fertility in women, and damage to adjacent organs such as the ureters are avoided. Complication rates after IRA are low, and postoperative bowel function is almost always good, averaging four semi-formed stools daily. After the IRA, the rectum remains at risk of cancer. Therefore, postoperative six-monthly surveillance of the rectum is mandatory.

An IPAA procedure removes the CRC risk almost completely but is more complicated than an IRA. It carries a higher morbidity and risk of complications. It often requires a temporary loop ileostomy, reoperations and longer hospital stays, night evacuation, and decreased fertility in women [27]. Bowel frequency is generally higher than that for an IRA. The risk of cancer does exist after IPAA as they may develop at the anastomosis or below. The pouch should be examined regularly postoperatively for adenomas [28].

The advantages of an IPAA with a lower risk of cancer must be balanced against the higher operative morbidity. Conversion after an IRA to an ileoanal pouch can be carried out when the patient is older [29]. An IPAA is the treatment of choice if the patient has a large number of rectal adenomas, for example, >20 adenomas, if there is the presence of adenoma with severe dysplasia, a colon with >1000 adenomas, or those with high-risk genotypes (e.g., codon 1309) [30, 31]. In patients with only a few rectal adenomas or with a polyp-free rectum, both options are possible although an IRA may be preferable. The decision can be made on an individual basis, considering preoperative sphincter function, patient compliance, and risk of desmoid.

Desmoid Disease

Desmoids are locally aggressive but non-metastasizing myofibroblastic lesions which occur with disproportionately high frequency in patients with FAP. Putative risk factors include abdominal surgery, positive family history for desmoids, and site of the mutation (mutations beyond codon 1444). In contrast to sporadic desmoid tumors, the majority of the tumors associated with FAP are located in the abdominal wall or intra-abdominally. The tumors can be diagnosed by CT scanning or MRI. Desmoids occur most commonly in the peritoneal cavity (Fig. 51.8) and may infiltrate locally leading to small bowel, ureteric, or vascular obstruction. These lesions may progress rapidly or may resolve spontaneously, their unpredictable nature making them difficult to treat [32]. Attempted surgical resection carries a high morbidity and mortality and usually stimulates further growth. The options for treatment are pharmacological (nonsteroidal anti-inflammatory drugs (NSAIDs) [33] and/or anti-estrogens), chemotherapy, surgical excision, or radiotherapy. Pediatricians treating children with extraintestinal desmoid tumors should consider the possibility of FAP in the family.

Chemoprevention

NSAIDs may be protective against colon cancer by inhibiting prostaglandin synthesis via their effects on cyclooxygenase (COX). Publications have shown a significant reduction in the number of rectal polyps in those patients taking the NSAID sulindac after colectomy. However, despite pro-

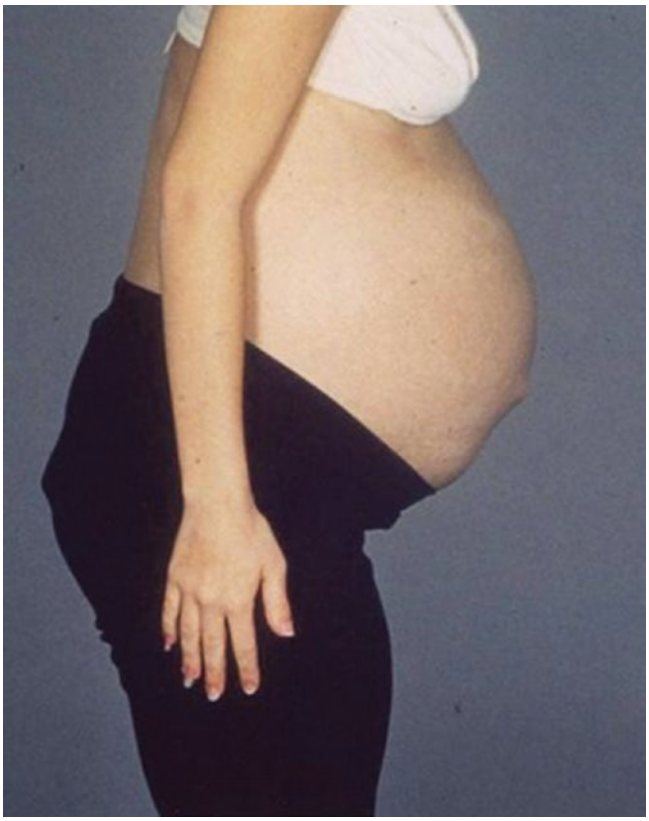

Fig. 51.8 Intra-abdominal mass from a desmoid tumor seen in a teenager with FAP

tracted drug use, the adenomas still progressed with case reports of rectal cancer. Sulindac administered before the development of polyps in genotype-positive adolescents did not prevent the development of adenomas.

Cyclo-oxygenase-2 (COX-2) enzyme is induced in inflammatory and neoplastic tissue. Selective COX-2 inhibitors generate fewer gastrointestinal-related side effects compared to the classical non-selective NSAIDs. One of these drugs (celecoxib) was found to reduce the number of colorectal adenomas by 28% [34] and also to reduce the number of duodenal adenomas [35]. Unfortunately, cardiovascular side effects have been reported in patients using selective COX-2 inhibitors. The safety and efficacy of celecoxib as a chemopreventive agent in pediatric population was first studied in a cohort of 18 children of ages 10–14 years with APC gene mutations and/or adenomas with a family history of FAP [36]. Celecoxib at a dose of 16 mg/kg/day, corresponding to an adult dose of 400 mg twice per day, was well tolerated and significantly reduced the number of colorectal polyps by 44.2% at 3 months (p = 0.01), but the cohort was small (n = 18). The largest randomized placebo-controlled chemopreventive study in children using celecoxib (n = 106) suggested the drug was well tolerated and there was a non-significant trend to slower progression of colorectal adenomas in the therapeutic arm compared to placebo [37]. Although NSAIDs cannot replace surgical treatment for colonic FAP, they may play a role in postponing surgery in patients with mild colonic polyposis or patients with rectal polyposis after prior colectomy. Other agents investigated but with so far inconclusive effect on polyp burden include vitamin C, oral calcium, and ornithine decarboxylase inhibitor difluoromethylornithine (DFMO) and eicosapentaenoic acid present in fish oil [38].

Prognosis

Genetic investigations, colonic surveillance, and prophylactic colectomy have impacted favorably on mortality in FAP-affected patients. Studies that evaluated the mortality of patients with FAP reported that surveillance policies and prophylactic colectomy have resulted in a reduction in the number of FAP patients that died from CRC. Currently, a greater proportion of deaths are attributable to extracolonic manifestations of the disease (desmoid tumors, duodenal cancer). Central registration in a family cancer registry and prophylactic examination lead to a reduction of CRC-associated mortality and ensure appropriate follow-up and patient support.

MYH-Associated Polyposis and Lynch Syndrome

MYH-associated polyposis (MAP) is characterized by the presence of adenomatous polyposis of the colorectum and an increased risk of CRC. Patients present with a variable number of polyps but no apparent extracolonic features. This autosomal recessive condition results from a compound heterozygote of the *mutY* human homologue (MYH) gene located on chromosome 1p. MYH polyposis should have no pediatric implications as colonic polyposis typically occurs in patients in their 40s, although cancer and polyps can occur at earlier ages. Surveillance colonoscopy in affected individuals should commence at age 25 years.

Lynch syndrome is an inherited condition with a high penetrance for the development of early CRC caused by an alteration in DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*). With no described pediatric complications, the syndrome is associated with colorectal, endometrial, ovarian, gastric, renal tract, and other cancers. The median age for the development of CRC in Lynch syndrome is approximately 44 years and is uncommon below the age of 25 years. Revised criteria (Amsterdam II or Bethesda criteria) exist to help identify affected families and enable genetic testing for the DNA mismatch repair genes in early adulthood. Once identified as at risk, colonoscopic surveillance should be undertaken from the age of 20 to 25 years.

Other Polyposis Syndromes

Gorlin syndrome is an autosomal dominant condition comprising upper gastrointestinal hamartomas and pink or brown macules in exposed areas such as the face and hands.

Turcot syndrome (also referred to as brain tumor-polyposis syndrome) is characterized by concurrence of a primary brain tumor (most often glioblastoma multiforme) and multiple colorectal adenomas. The number of adenomas is often not high, but many of the reported patients have been adolescents. Patients with a polyposis syndrome and neurological symptoms should undergo thorough neurological examination and investigation for possible brain tumor.

In patients with long-standing inflammatory colitis, inflammatory polyps (pseudopolyps) may develop. Inflammatory polyps are of no significance and have no malignant potential.

The Role of a Polyposis Registry

There are numerous advantages to integrating a family into a cancer/polyposis registry. Polyposis registries are established across the world. A polyposis registry enables registration of polyposis patients and family members accessing counseling and genetic testing and initiation and coordination of screening of family members at risk of a polyposis syndrome. By increasing the rate of diagnosis of FAP and other polyposis syndromes, and enabling earlier diagnosis, there is proven improved survival of patients registered, almost certainly attributable to the improvement in organization and coordination of patient screening [39].

Other Tumors of the GI Tract (Excluding the Stomach and Hepato-biliary)

Gastrointestinal Stromal Tumors

Gastrointestinal stromal tumors (GISTs) are a unique variety of mesenchymal tumors that have recently been described. They are very uncommon among children and adolescents, and their true incidence is not well known due to the small number of described cases.

The overall incidence rates of GIST are reported to range between 6.5 and 14.5 per million per year [40–42]. The UK National Registry of Childhood Tumours showed an annual incidence of 0.02 per million children below the age of 14 years [43].

GISTs were described for the first time in 1990 and probably arise from neural interstitial cells of Cajal. Its molecular oncogenetics derives from mutations in the KIT oncogene (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue) that encode for class III receptor tyrosine kinases and platelet-derived growth factor receptor alpha (PDGFRA), which are expressed in GIST patients. About 0–10% of pediatric GISTs have an oncogenic KIT mutation [44–46], and only two patients were reported with a PDGFRA mutation to date [47, 48] which reflects probably other downstream activation pathways other than c-Kit mutation. A small subgroup of syndromic GISTs were found to have common germline mutations encoding the succinate dehydrogenase (SDH) gene [48, 49]. Recently, another molecule, IGF1R (insulin growth factor receptor 1), was found to be significantly expressed in a series of 17 GISTs including one pediatric GIST, both by Western blot analysis and immunohistochemistry, and that it seems to be a new attractive pathway for this complex oncogenic process [50].

Immunohistochemically, these tumors stain positive for vimentin (CD-117, a *c-kit* proto-oncogene protein) and CD-34—and stain negatively for smooth muscle actin [51]. Since the use of CD117 staining in 2002, 82% of mesenquimatous tumors were considered to be GISTs, so its true incidence rose since there, with a proportional decline of smooth muscle tumors cases [52]. Histologically, GISTs are composed either of spindle-shaped cells, epithelioid cells, or a combination of both, being spindle-shaped type slightly more frequent in a large analysis of 99 of 113 pediatric cases [53].

GISTs can present as sporadic or inherited cases, and the latter can be inherited through familial autosomal dominant fashion or as syndromic tumors, named as the Carney triad.

Carney Triad

GIST can occur rarely in association with other tumor syndromes. The association of gastric leiomyosarcoma, extra-adrenal paraganglioma, and pulmonary chondroma was first described in 1977 and subsequently named the Carney triad [54]. It affects predominantly females, and it has been described as a chronic benign-course tumor that comprises recently also adrenocortical tumors and esophageal leiomyoma [55–57]. Neither KIT, PDGFRA, nor SDH mutations were found in patients with the Carney triad. In 2002, Carney and Stratakis distinguished an inherited tumor syndrome comprising GIST and paragangliomas which have common germline mutations encoding SDH subunits B, C, and D, and it was termed Carney–Stratakis dyad [58].

Familial

Familial GIST cases are very rare, and they are autosomal dominant inherited. They differ from Carney dyad as they are heritable germline KIT mutation tumors. Occurrence of

multiple tumors can be observed both in syndromic [59–64] and in sporadic GIST. Familial GISTs do not have female predominance, and the main location is the small intestine, in contrast to sporadic GIST where the stomach is the main affected place [61, 65]. Other phenotypic characteristics associated with familial GIST include mastocytosis [66], dysphagia [67, 68], cutaneous hyperpigmentation or urticaria pigmentosa [59, 61, 64, 69, 70], and recurrent small intestinal diverticular perforation [68]. Familial GIST does not develop early in life, as the youngest patient in a GIST family described to this date had symptoms at 18 years old [70].

Sporadic

A considerable number of articles describing children and adolescents with sporadic GIST have been published [71–73]. Sporadic GISTs have been described in newborns [72–75], but the majority of cases become symptomatic in the second decade of life. There is a strong female predominance with a male-to-female ratio of 1:2.5.

The majority of pediatric GISTs are located in the stomach (mainly in the antrum) [45, 72–74, 76–80], but it can be found virtually in every digestive tube segment as well as mesentery, omentum, and abdominal wall [81–84].

Clinical presentations vary from upper gastrointestinal bleeding with anemia and anemia-related symptoms to nonspecific symptoms such as loss of appetite, poor feeding, abdominal pain, abdominal distension, nausea, vomiting, constipation, epigastric discomfort, diarrhea, and intestinal obstruction [53]. Sometimes they are incidentally discovered in a routine abdominal examination as a palpable mass, suggestive for a more advanced stage of the disease, or at ultrasound [53].

GISTs generally have a nodular growth pattern, with frequent submucosal spreading. Surface ulceration may result in acute or chronic bleeding which promotes clinical investigation (Fig. 51.9). Gastrointestinal contrast series denote solitary or multiple nodular round filling defects. Even without metastases on presentation, submucosal spreading can occur, so endosonography can be a very useful diagnostic resource [44, 80, 85–87]. Other image tools include abdominal and thoracic CT or MRI to perform disease staging. Metastases on diagnosis are not frequent, but they may occur in the liver (most commonly), lymph nodes, peritoneum, and mesentery. Even without metastasis on diagnosis, these are very frequent places for recurrent disease [47, 72, 76, 77, 87].

Fluorodeoxyglucose positron emission tomography (FDG-PET) seems to be a sensitive diagnostic tool, particularly to monitor treatment response. The final diagnosis is based on histology and immunohistochemistry. Tissue samples can be obtained either by biopsy or by tumor resection, which in many cases of small localized disease, is usually the only procedure needed [88].

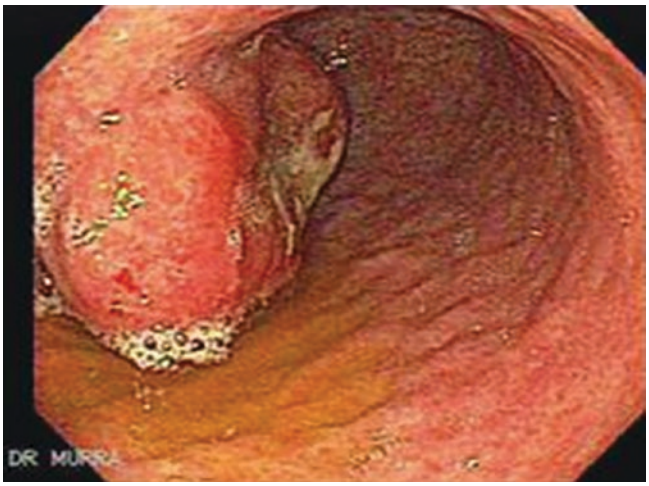

Fig. 51.9 GIST in the stomach. GISTs gastrointestinal stromal tumors

There are no algorithms for children GIST workup as well as for the treatment. As in adults, total resection with microscopic free margins is the gold standard treatment. Adjunctive therapy from conventional cytotoxic chemotherapy had no positive results. Localized disease is usually cured by surgery, and it can be performed more than once in recurrent disease or in incomplete primary resection. Metastases should undergo surgical treatment as lymph node dissection and resection of hepatic-affected areas [88].

Recently, there are increasing cases of children treated with RTK inhibitors (imatinib and sunitinib), based on adult experience. They may be considered in high-risk patient such as metastatic diffuse disease or when the primary tumor cannot be fully resected. Despite the fact that there are no randomized trials in children or consensus on imatinib dosage, a starting dose of 400 mg/m^2 once daily might be suggested with a maximum dose of 400 mg bid = 2 times daily [53].

In children, prognosis depends on the results from surgery of the main tumor lesion and the presence of metastatic lesions, especially if they are not amenable to complete resection [44, 76, 77, 89–91]. Recurrences are frequent and may occur many years following diagnosis, but it seems that mortality from GIST is low, despite tumor recurrence and development of metastases.

In summary, the pathogenesis, clinical course, and prognosis of GIST in children are currently insufficient due to the small number of reported cases. Future research based upon controlled randomized trials should be encouraged to inform about optimal management in this particular population.

Gastrointestinal Autonomic Nerve Tumors

Gastrointestinal autonomic nerve tumors (GANT) are variants of GIST. Diagnosis is based on electron microscopic studies showing neural differentiation in contrast to GIST, and the number of pediatric patients with GANT is limited to a few cases [92–97].

Inflammatory Pseudotumors

Inflammatory pseudotumors are solid tumors composed of spindle cells, myofibroblasts, plasma cells, and histiocytes that can occur in every organ system during childhood [51, 98, 99]. Lung is the most common location, whereas occurrence in the gastrointestinal tract is rare, the majority involving the stomach [100] (Fig. 51.10). They are commonly described as plasma cell granulomas or inflammatory myofibroblastic tumors. Abdominal pain is the most common presenting symptom but may also present with dysphagia, intestinal obstruction, and iron-deficient anemia. It affects mostly girls, as reported in the literature [98].

Like the majority of gastrointestinal tract tumors in childhood, complete surgical resection is the treatment of choice. Sporadic trials of nonsteroidal and steroidal anti-inflammatory medications have been reported for the treatment of large and unresectable masses. Its success rate seems to be variable [101]. Recurrence rates are reported between 18% and 40% and are more common in extrapulmonary lesions. Multiple recurrences are associated with a higher rate of malignant transformation with an overall mortality of 5–7% [51, 102, 103].

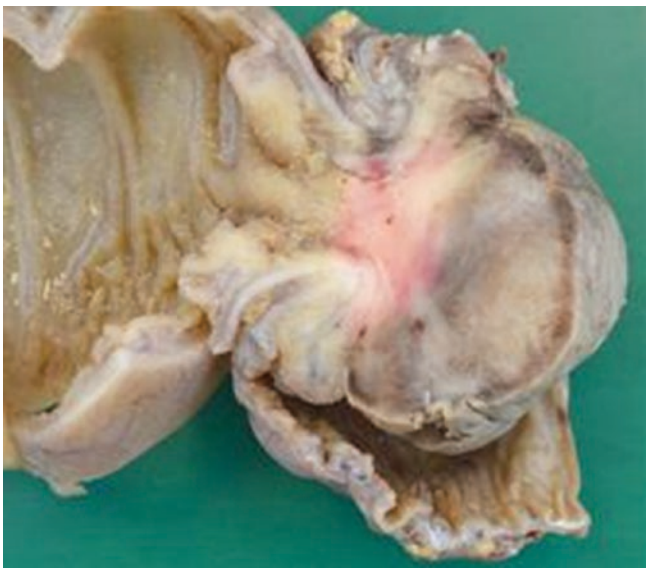

Fig. 51.10 Inflammatory pseudotumor

Sarcomas

Soft tissue sarcomas account for 7% of all childhood malignancies, and only 2% of this group have digestive involvement [104, 105]. Sarcomas are smooth muscle-derived neoplasms that occur anywhere along the gastrointestinal tract, more often involving the stomach and small intestine (leiomyomas) (Fig. 51.11). In the stomach, these can be confused with the appearance of an umbilicated lesion typical of an ectopic pancreas. The malignant form (leiomyosarcoma) is more common in the jejunum [106, 107]. The definition of malignancy usually is based on mitotic index at histologic examination (more than ten mitoses per high-powered field), but in children, it is not common to see a high mitotic index that enables the adult classification of leiomyosarcoma [51]. Nearly 40% of these present with less than five mitoses per high-powered field, so pathologic features must also include tumor size, cellular atypia, tumor necrosis, and myxoid change [108].

Clinical presentation occurs early on life, with over half of the leiomyosarcomas occurring in the newborn period, but they can present throughout childhood and adolescence. The clinical signs vary from an incidental mass finding to active GI bleeding associated with anemia signs or acute perforation. They can also be the cause of recurrent intussusception [109–112]. Rare presentations include cough and dysphagia (if esophageal location) and oral cavity swelling [113, 114].

Symptomatic lesions may also present concurrently in patients with neurofibromatosis and in children with human

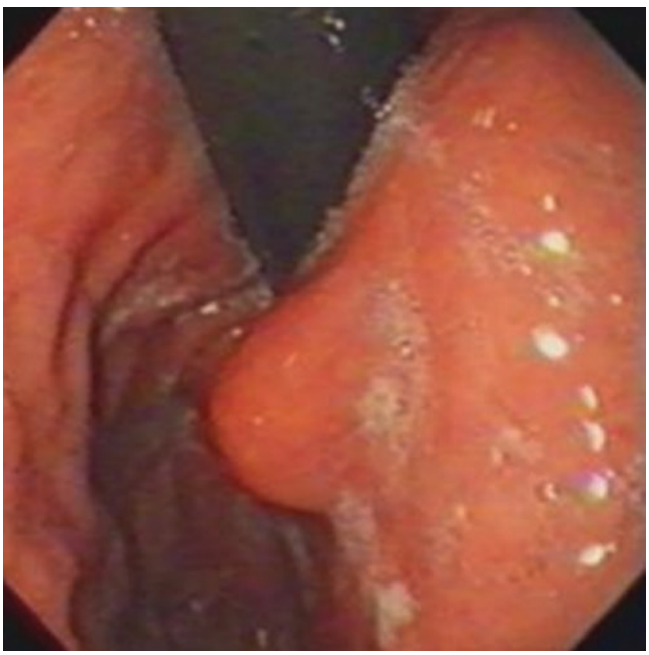

Fig. 51.11 Leiomyoma of the stomach

immunodeficiency virus (HIV) [115–117]. The goal of treatment is complete resection as pediatric leiomyosarcomas are less likely to be found with metastases at diagnosis [118], and the use of adjuvant chemotherapy and radiotherapy remains experimental. Long-term outcomes for leiomyosarcomas are related with the histologic type of the tumor and with successful surgical excision.

Carcinoid Tumors

Carcinoid tumors in children are quite rare, and their true incidence is unknown. The occurrence of carcinoid tumors in children was recently reported as approximately 0.1% of all pediatric cancers [71, 119]. These tumors are more prevalent in school-aged children, but younger patients have been reported. An overall female-to-male ratio of 3:1 has been noted [119]. Despite their rarity, carcinoid tumors have been described in all portions of the gastrointestinal tract from the stomach to the rectum. The most common location is the appendix, followed by involvement of the small intestine and rarely the colon and rectum [120]. Reports have also documented the presence of carcinoid tumors in gastrointestinal duplication cysts and Meckel's diverticulum [121–123]. The diagnosis is often made through the incidental pathologic findings within an appendectomy specimen. However, these tumors may present with clinical signs of hematochezia or anemia from chronic gastrointestinal blood loss, right-lower quadrant abdominal pain, and small bowel obstruction-associated signs [119, 121, 124]. The vast majority of carcinoid tumors are benign. Occasionally, they may be locally invasive, especially if originating from the colon or small intestine. Few reports have noted their malignant transformation in children, with metastasis to the liver, lung, and bone [119]. Serotonin hypersecretion signs known as carcinoid syndrome with cutaneous flushing, diarrhea, asthma-like respiratory distress, and right-sided cardiac failure are quite rare [121, 124, 125].

Incidental gastrointestinal carcinoid tumors which are less than 2.0 cm in diameter and without evidence of metastasis are successfully treated with complete resection, which should include tumor-free margins. For those with evidence of serosal penetration or local extension, a bowel resection with associated mesenteric resection is required, along with abdominal surveillance for metastatic disease [51]. Patients with tumors exceeding 2.0 cm in size should undergo thorough evaluation for possible metastases to the liver, lung, and bone. Serum 5-hydroxyindoleacetic acid (5-HIAA) levels may act as a serologic marker of disease, but are not present in all metastatic cases. Serum chromogranin A is more reliable as a marker and does not require metastases for this to be an abnormal result. Abdominal and thoracic CT or MRI and 99m-technetium bone scan should be considered in patients with metastatic disease and/or bone pain. Children with metastatic disease often respond poorly to cytotoxic chemotherapy, but they can benefit from the administration of octreotide for symptomatic relief [119].

Gastroenteropancreatic Neuroendocrine Tumors

Pediatric gastroenteropancreatic neuroendocrine tumors (GEP-NETs) have traditionally been classified into two groups: carcinoids, which are subdivided by their origin into tumors of the *foregut* (lung, thymus, stomach, duodenum); *mid-gut* (jejunum, ileum, appendix, right colon), and *hindgut* (left colon, rectum); which were previously discussed; and pancreatic tumors [126]. A more useful classification was proposed by the World Health Organization in 2000: well-differentiated NETs, which show benign behavior and well-differentiated neuroendocrine carcinomas, which are characterized by low-grade malignancy and poorly differentiated NEC of high-grade malignancy [127].

These tumors can be *functional* or *nonfunctional*. Functional tumors have clinical symptoms caused by hypersecretion of hormones such as gastrin, while nonfunctional tumors can produce clinically silent hormones such as pancreatic polypeptide. The functional tumors are named according to the hormone responsible for the clinical syndrome, such as gastrinoma causing Zollinger–Ellison syndrome and insulinoma causing hypoglycemic syndrome. NETs can either be sporadic or occur as part of familial syndromes such as multiple endocrine neoplasia (MEN) I and II, von Hippel–Lindau (VHL) syndrome, and neurofibromatosis type I (NF-I) [128].

GEP-NETs are very rare in children. Tumors with gastroenteropancreatic hormone production include amine precursor uptake and decarboxylation tumors (APUDomas), extrapancreatic gastrinomas, and vasoactive intestinal polypeptide tumors [129, 130]. Pancreatic NETs account for approximately 30% of pancreatic tumors in patients younger than 20 years. While insulinomas are the most common in adults, gastrinomas are more common in children and affect more frequently the stomach. Hypersecretion of gastrin by gastrinomas causes recurrent or ectopic peptic ulcers (Zollinger–Ellison syndrome) or malabsorption diarrhea [128]. Non-gastrinoma neuroendocrine tumors can occur in any part of the pancreas [131]. Metastases are present in 60–80% of patients at initial diagnosis of gastrinoma, with the liver and lymph nodes being the most common sites of metastatic disease [132]. There is limited information in the pediatric literature regarding the imaging findings of NETs

as well as treatment options. PET scan with octreotide labeling may help in diagnosis. Prognosis of this rare entity depends on disease extension and presence of metastatic disease. A recent study from a European registry showed an overall survival of 100% when complete surgical resection was performed in patients with localized neuroendocrine neoplasm (NEN) in contrast to poor survival rate associated with metastatic disease [133].

Adenocarcinoma

Progression to adenocarcinoma from polyposis syndromes such as FAP and the HNPCC is dealt with the above.

Colorectal cancer's prevalence in children is very low with fewer than 500 pediatric cases reported in the literature and a few articles dealing with this as reviews [134–138]. Compared to adults who have no predetermining diseases, in children, there is usually preexisting polyposis or, rarely, colitis.

Pathogenesis

Colorectal carcinoma is due to sequential genetic mutations, commonly known as the adenoma–carcinoma sequence. Progress to malignancy happens due to the coexistence of four or more genetic problems which can include mutational activation of oncogenes and inactivation of tumor suppressor genes.

Pathology

Colonic carcinoma in children is usually cecal or right-side colonic disease, with 50% in the ascending and transverse colon. If colonic cancer penetrates the bowel wall, it can spread to regional lymphatics and subsequently to distant lymph nodes and metastasize to the liver, lungs, and vertebrae. Cases are staged according to the degree of local spread and presence or absence of transmural penetration, the degree of lymph node involvement, and the presence of distant metastases. In a review of younger cases with colonic cancer, 82% of these cases had either distant metastases, lymph node involvement, or transmural penetrating disease, significantly higher than adult cases.

In adults, the CRC is that of a moderate to well-differentiated adenocarcinoma. In children, this is a more aggressive type, with more proportion of tumors with mucinous (30%) and signet-ring (10%) histological appearances, hence with a poorer prognosis—this probably leads to the poorer prognosis at presentation in children (Fig. 51.12).

Clinical Presentation

Abdominal pain is the main presenting symptom in the majority of cases—other symptoms include weight loss, intussusception, vomiting, rectal bleeding, and altered bowel habit. The site of the lesion is important with constipation, obstruction, and bleeding more common with left-sided disease and, more commonly in childhood, right-sided disease which may not present with obstruction until the cancer is larger and a mass is palpable.

Diagnosis is by most usefully achieved by ileocolonoscopy in the majority (Fig. 51.13), although the lesion

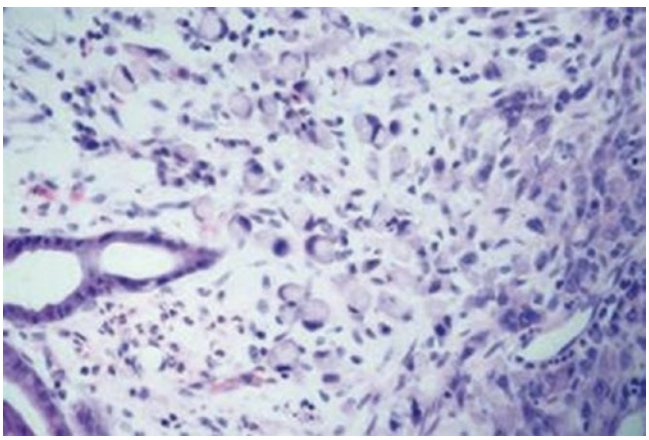

Fig. 51.12 Histological appearance of signet-ring adenocarcinoma

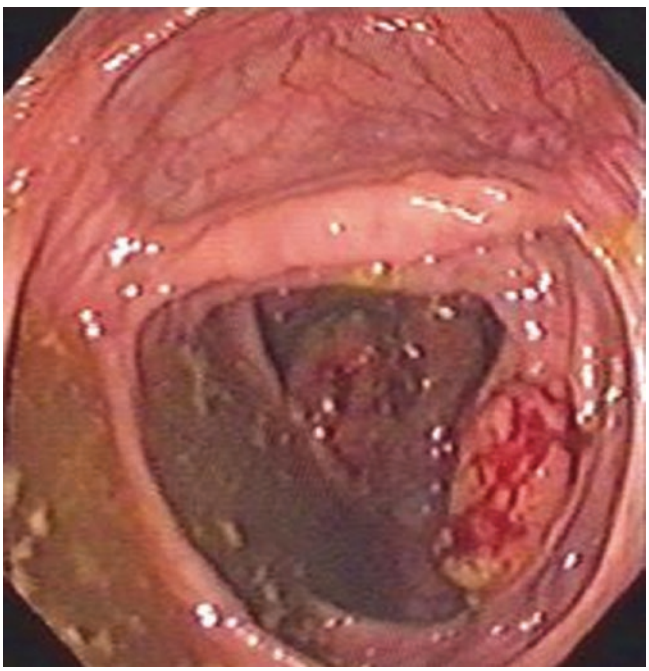

Fig. 51.13 Colonoscopic appearance of rectosigmoid adenocarcinoma

can also be identified by other diagnostic techniques including wireless capsule endoscopy, ultrasound, CT colonography, or MRI. Although serological markers such as carcinoembryonic antigen (CEA) are available, their role in establishing this rare diagnosis in children has not been fully evaluated.

Treatment/Prognosis

Surgery is the most effective treatment for colonic carcinoma, with complete resection of the primary lesion and regional lymph nodes being the main aim; however, total clearance and resection are often not possible in children due to the advanced stage at presentation. Chemotherapy for metastatic disease has poor results, and the role of radiotherapy or combination therapies grouping childhood cases is hampered by limited numbers presenting precluding viable randomized studies.

Sadly the prognosis for children with colon cancer is poor with 5-year survival rates of 10–20% [134–141]. These poor results are due to a combination of aggressive phenotype and delayed presentation.

Lymphoma

Epidemiology and Classification

In the developed world, lymphoma accounts for 10% of all cancers in children under 15 years of age—however, it is an unusual GI condition and would need a predisposing condition in most cases, for example, celiac disease or isolation by distance (IBD). Nevertheless, cases occur without these prerequisites [142, 143].

Etiology

Non-Hodgkin's lymphoma (NHL) usually presents in association with immunodeficiency syndromes—inherited (e.g., ataxic-telangiectasia, severe combined immunodeficiency (SCID)) and acquired (e.g., HIV infection)—and also with immunosuppressive drug regimens such as those encountered after liver transplant [144]. Epstein–Barr virus (EBV) may play a role in disease pathogenesis especially in Burkitt's lymphoma, although malaria coinfection may be important in promoting B-cell activation as part of the process. Other conditions that give rise to chronic mucosal inflammation have also been linked to lymphoma including *Helicobacter pylori*. T-cell NHL is associated with celiac disease, while both lymphoma and adenocarcinoma are associated both with Crohn's disease where the lesion is typically in the small bowel and with ulcerative colitis [142, 143].

Pathology

Intra-abdominal NHL in children typically is of an undifferentiated histological type—only around 50% have a primary intestinal origin—and the most common intestinal primary sites are the distal ileum, cecum, and appendix. Bone marrow involvement occurs in up to 40% of undifferentiated lymphoma, while central nervous system involvement is uncommon. In Burkitt's lymphoma, 60% can have abdominal disease.

Clinical Presentation

Disease may present with nonspecific symptoms such as abdominal distension, nausea, vomiting, altered bowel habit, and abdominal pain. Lymphoma in the GI tract is typically localized to the distal ileum and cecum and would present as a mass or occasionally as an ileo-cecal intussusception manifesting with obstructive symptoms or bleeding.

Endoscopy is important in diagnosis (Fig. 51.14), and when the tumor is suspected but is outside the reach of conventional endoscopes, then wireless capsule endoscopy (Fig. 51.15) and balloon enteroscopy can be useful tools in identifying lesions and then if necessary biopsy of these lesions and tattooing, thereby accurately localizing them in case of the absence of serosal abnormality for future surgical

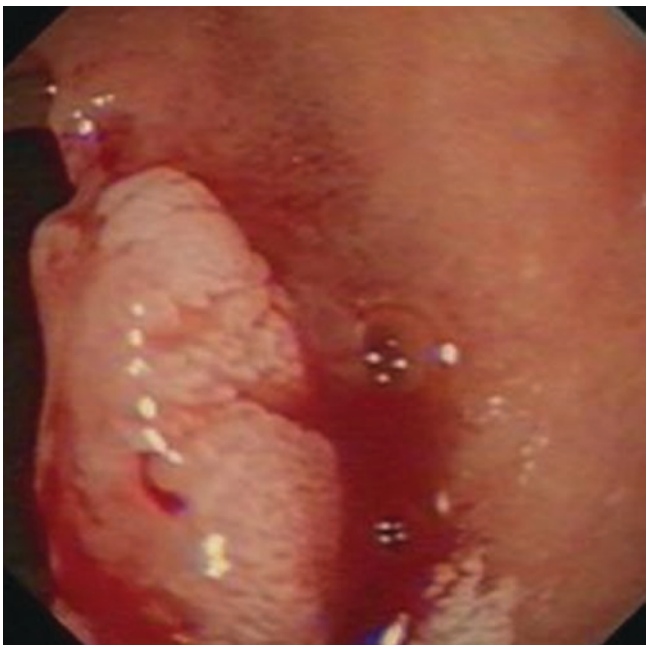

Fig. 51.14 Endoscopic appearance of small bowel lymphoma

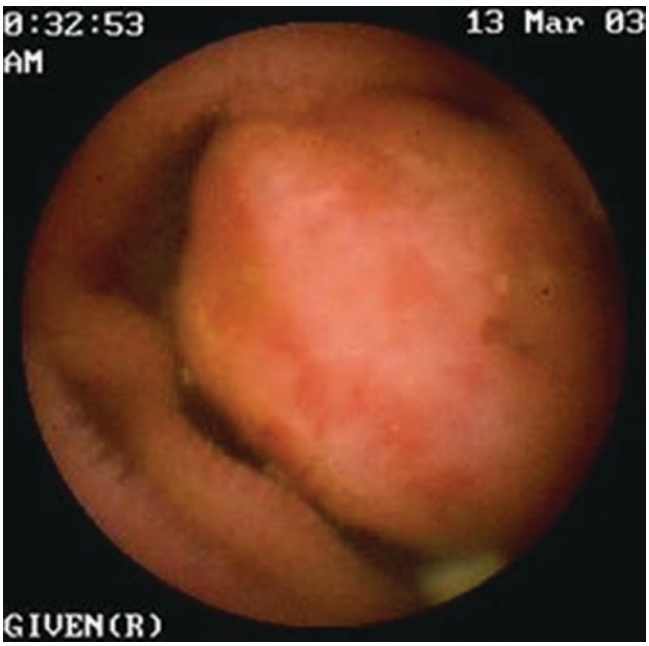

Fig. 51.15 Lymphoma in the mid-small bowel identified by wireless capsule endoscopy

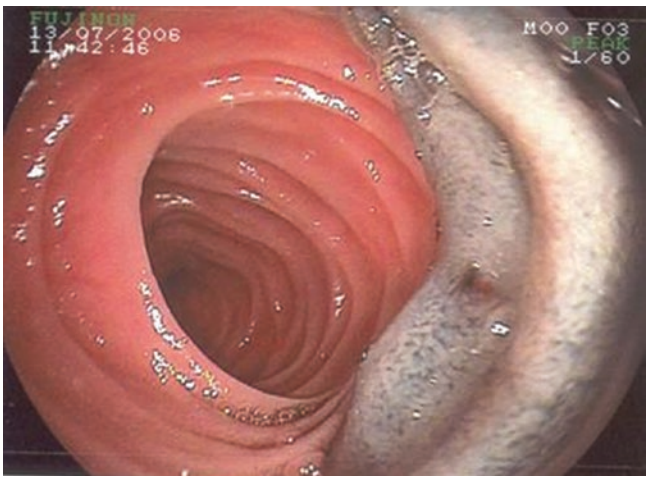

Fig. 51.16 Biopsy and then tattooing for identification of the exact site of a small bowel tumor by double-balloon enteroscopy prior to surgery

resection (Fig. 51.16). The diagnosis of NHL depends on histological examination with immunophenotyping and cytogenetics. Staging of disease with chest and abdominal imaging, lumbar puncture, and bone marrow examination is also required and may allow treatment to start if there is a delay in undertaking a laparotomy.

Treatment and Outcome

Surgical resection of local disease is indicated where it is possible, although such fully resectable disease accounts for only 60–75% of cases [144]. The high mortality associated with intestinal perforation in more advanced disease has led to debate as to the role of partial resection as opposed to simple biopsy to establish histological type. Subsequent chemotherapy is required in all cases. For more localized disease, the overall outcome is relatively good. In 30% of childhood, NHL cases with localized disease cure rates as high as 95% have been reported following combined surgical resection and combination chemotherapy [144].

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Fecal Microbiota Transplantation in Children

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Valentina Giorgio, Elisa Blasi, and Giovanni Cammarota

Abbreviations

CDI	*Clostridium difficile* infection
FMT	Fecal microbiota transplantation
GVHD	Graft-versus-host disease
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
MDRI	Multidrug-resistant organism infections
SAEs	Serious adverse events

Introduction

Humans are inhabited by a huge number of microbes, most of them being located in the gastrointestinal tract: the intestinal microbiota. This is not a simple reserve of microorganisms; it should be considered a real organ instead [1, 2]. Plenty of research is ongoing on this matter, but its composition is not yet fully known. *Bacteroidetes* and *Firmicutes* are the most represented phyla, having on their side archaea, viruses, fungi, and protozoa [3].

Gut microbiome is involved in a large number of physiologic functions including modulation of local and systemic immunity, regulation of metabolic pathways, and development of a barrier against exogenous agents. A solid scientific evidence suggests that dysregulation of intestinal microbiome homeostasis can lead to development of both digestive and extradigestive diseases, ranging from gastrointestinal infections to inflammatory bowel disease (IBD), colon cancer, metabolic syndrome, irritable bowel syndrome (IBS), and allergies [4].

Theoretically the reconstitution of a "healthy" intestinal microbiota represents a valid approach for the management of diseases related to microbiota dysregulation. Antibiotics, probiotics, and prebiotics are currently the most widely used therapeutic options in children. In adults, fecal microbiota transplantation (FMT) has already demonstrated undoubted efficacy in the management of recurrent *Clostridium difficile* infection (CDI), and it is already considered a therapeutic strategy for other diseases associated with intestinal microbiota imbalance [5]. At the moment there is paucity of data on FMT in children and the associated microbiome changes.

It is well known that microbiome of a child, especially in the very young, differs from that of an adult, both in terms of initial colonization of the infant gut and in terms of dynamic microbiome development over the years, until the stage for a relatively stable microbiome in the healthy adult [6].

Fecal Microbiota Transplantation in Children

FMT is the infusion of feces from a healthy donor to a sick recipient for the treatment of a specific pathology, in order to modify the microbial composition and reduce gut dysbiosis [5]. Transfer of human fecal product modifies microbiome composition shifting it toward the healthy donor, increasing recipient fecal microbial biodiversity, short-chain fatty acid, and secondary bile acid synthesis [7]. These changes can persist up to 6 months following FMT [8].

In children, especially in the very young age, the intestinal microbiome is known to differ from that of adults. Very little data has been published in recent years characterizing associated microbiome changes with FMT in pediatric populations. Microbiome changes have been reported in a

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20- month-old boy [9], with decreased diversity of the microbiome prior to FMT—abundance of *Proteobacteria*— and increased diversity after FMT, increasing of *Bacteroidetes*, similarly to what has been already described in adults [7]. In a case series of older children with CDI and with or without IBD, increased microbiome diversity after FMT has also been described [10].

In general, microbial signatures demonstrating decreased gut microbiota and phyla diversity have been shown to predispose individuals to CDI [5].

Anyhow, the administration of FMT products requires a rigorous screening process in order to minimize the risk of donor-derived infection (DDI). Consensus recommendations have been published for adults and also for children, jointly by the North American (NASPGHAN) and the European (ESPGHAN) Society for Pediatric Gastroenterology, Hepatology and Nutrition [11]. A combination of screening questionnaires (to exclude high-risk donors), donor serologic testing for transmissible pathogens (e.g., syphilis and HIV), and donor stool tests to reduce the risk of DDI (e.g., stool enteric pathogen culture, CDI testing, and *Norovirus* and *Adenovirus*) are requested. Institution-specific protocols are also available in FMT centers.

Pediatric Fecal Microbiota Transplantation: Clinical Indications

Clostridium difficile, more recently termed *Clostridioides difficile*, is a spore-bearing gram-positive anaerobic organism responsible for significant morbidity and mortality among children and adults. In children, the incidence of CDI has significantly increased in the last 20 years. Some studies have reported an almost twofold increase in pediatric CDI hospitalizations between 2001 and 2006, and a 12.5-fold increase in overall CDI incidence per 100,000 children [12].

Risk factors for CDI include antibiotic use in the previous 4 weeks, solid organ transplant, and the presence of gastrostomy or jejunostomy (G or J) tube. CDI diagnosis is often challenging because of high carrier rates for *Clostridioides difficile* in children less than 2 years of age [11].

Recurrence following CDI occurs in 11–20% of pediatric patients [12]. Management of recurrent CDI is not well studied in children, with evidence mostly inferred from adult studies. FMT has been shown to be more effective than antibiotics for recurrent CDI in adults. Cammarota et al. [13] showed a cure rate of 90% in patients treated with FMT compared with 26% rate in those treated with vancomycin. van Nood et al. [14] showed a resolution rate of 81% after FMT compared with 31% after vancomycin alone. However no randomized controlled trials (RCT) in children have been performed yet. There is also paucity of pediatric large case series up to now. A multicenter retrospective cohort study by Nicholson et al. on 372 children—between the ages of 11 months and 23 years old—affected by recurrent CDI [15], reported that FMT was effective in 81% cases, demonstrating CDI resolution at 2 months following a single FMT. It has to be noted that 32% of patients in this cohort had IBD. Multiple case series and reports have shown therapeutic success of FMT in pediatric RCDI, regardless of the mode of delivery.

Nonetheless, there is very limited data on FMT treatment in pediatric IBD, and no RCT is available to date. The evidence in pediatrics is limited to case series and individual reports. In a report of ten children, IBD clinical remission was described in three of eight UC patients and two of two Crohn's disease (CD) patients after eight doses of FMT [16]. In a prospective study, Goyal et al. [17] reported a clinical response in 57 and 28% at 1 and 6 months after a single FMT in 21 IBD patients. Although responders showed an increase in diversity of microbiome 1 month after FMT, at 6 months these changes returned to baseline conditions. Other reports are available in literature demonstrating similar efficacy and transient response.

A separate mention needs to be done for very young children who represent a unique population, both given their dynamic microbiome profiles and their peculiar and changing physical size. The youngest reported child receiving FMT for CDI is a 13-month-old infant [18]; few other reports of cases under the age of 3 years have also been published. All cases were successful, but in this population, clinicians should be very careful about the choice of patient for FMT, given the high rate of asymptomatic carriage of *Clostridium difficile* in very young children, as largely specified by the joint NASPGHAN and ESPGHAN recommendations [11].

FMT has also been explored as a therapeutic strategy for the management of IBS and chronic idiopathic constipation. Available data are limited to the adult population and small numbers.

FMT has been successfully used in multiple reports to decolonize adult patients with multidrug-resistant organism (MDRO) infections, including vancomycin-resistant *Enterococcus* and *Klebsiella pneumoniae*. However, data regarding the overall efficacy of FMT in this setting remains limited, and no pediatric data is available so far.

FMT has been proposed as a mechanism to restore gut microbial diversity and decrease complications following hematopoietic stem cell transplant and GVHD. However, there are no pediatric data to date, and while the prospect of FMT for refractory gut GVHD remains promising, additional investigation is needed.

Potential therapeutic applications of FMT have been further explored in the treatment of a wide range of illnesses, including allergic colitis and behavioral and gastrointestinal manifestations of autism spectrum disorder [19, 20], but no robust conclusion can be designed in these two fields of interest.

Up to now, the only indication for which FMT can be used without an investigational new drug application from the Food and Drug Administration is CDI. When children with CDI may need FMT, guidelines may be the following: *(i)* in recurring or relapsing CDI, with at least three episodes of mild–moderate infection and failing a 6–8-week vancomycin taper or at least two episodes of severe CDI resulting in hospitalization and associated with significant morbidity; *(ii)* in moderate CDI not responding to standard therapy for at least a week; and *(iii)* in severe CDI with no response to standard therapy after 48 h. Children with CDI who may need FMT most often are affected by mild–moderate recurring or relapsing CDI.

Pediatric Fecal Microbiota Transplantation Safety Profile

Although the long-term safety of FMT has not yet been established, serious adverse events (SAEs) are overall rare. Among children, a multicenter retrospective cohort study of 335 patients receiving FMT for CDI demonstrated SAEs in 4.7% of subjects in the 3-month period after FMT [15]. Among children, data on the safety of FMT among immunocompromised hosts are scant, and no AE has been reported on four immunocompromised pediatric patients [12]. In general, while available studies to date suggest that FMT is safe and effective among immunocompromised individuals, additional studies are needed to characterize the safety and efficacy of FMT among immunocompromised children.

Conclusions and Future Perspectives

Up to now the only indication for which FMT can be used without an investigational new drug application is CDI also in pediatric populations. Nevertheless, while studies about FMT in children hold a great promise for the treatment of recurrent CDI, and possibly IBD, some concerns should be raised specifically pertaining to pediatric FMT. The first concern is about the stool donor: most studies on FMT in children have used an adult donor, even if it is well known that children, especially if in the first years of their lives, have a developing gut microbiome that correlates with development of the immune system and other physiological functions [21]. It should be further explored whether the transfer of an adult microbiome to a developing microbiome in a child may predispose to altering immune aging and developing immune-related complications [22] (Fig. 52.1). Currently there have been no studies with long-term follow-up of FMT in children to provide answers. Technical aspects for FMT in children are also unanswered. The optimal amount of stool to be used for children of varying sizes and best mode of delivery still needs to be addressed. Moreover, different donor screening protocols for infection should be used for FMT in children, who are less likely than adults to have been exposed to such infections as cytomegalovirus.

In conclusion, it is already known that FMT is an effective treatment for recurrent CDI in adults. In children data is still limited, but initial reports seem encouraging that FMT is effective for recurrent CDI as well. FMT seems to be safe at least in the short term in pediatric IBD. Nevertheless, clinicians approaching FMT in a child should keep clear in mind that manipulation of the intestinal microbiome has the potential to have implications on both gastrointestinal and non-gastrointestinal sites, especially when manipulating the developing microbiome of a child that is particularly vulnerable. Further studies are awaited on pediatric populations in the near future.

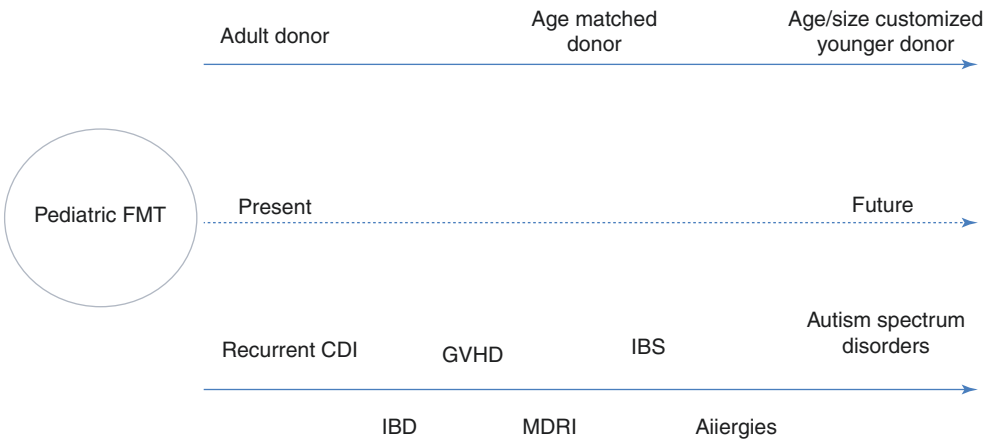

Fig. 52.1 FMT in children, present and future

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Prebiotics in Pediatrics

53

Francesco Savino

Introduction

The concept of prebiotics was first suggested by Gibson and Roberfroid in 1995 in their scientific publication *Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics*. In the decades that followed, scientific discussions about prebiotics tended to focus on identifying substances that targeted health-promoting groups of bacteria in the gut, in particular bifidobacteria and lactobacilli [1–3].

Although the original term was approximately defined, subsequent publications suggested that the fructans, namely, oligofructose and inulin, fructo-oligosaccharides (FOS), and lactulose, and the galactan, galacto-oligosaccharides (GOS), were the only established prebiotics at that time [4]. Early research mainly focused on those few substances (Table 53.1).

However, by the early 2000s, the development of the next generation of rationally selected prebiotics had been proposed [5]. Researchers suggested that resistant starch (RS), milk oligosaccharides and pectin, and other fiber components had prebiotic potential [6, 7]. These have now been classified as new prebiotics [5] (Table 53.2).

Research on the development of intestinal microflora in newborn infants showed that it was strictly related to the feeding method they received [8].

Breastfed infants, unlike bottle-fed ones, were shown to have an intestinal ecosystem that was characterized by a strong prevalence of bifidobacteria and lactobacilli [9, 10].

There are numerous substances present in human milk, and data have been published that show that one of the main components, oligosaccharides, has a clear prebiotic effect. They are only partially digested in the small intestine, and when they reach the colon, they selectively stimulate the development of bifidogenic flora. Studies have also confirmed that human milk oligosaccharides were the first prebiotics in humans. This has been recently proved both by the characterization of oligosaccharides in the feces of breastfed infants and by studying intestinal microflora using new molecular analysis techniques [11].

The oligosaccharide content and concentration in breast milk is a dynamic process, just like other macronutrients, and it has been impossible for the industry to mimic nature. However, even if the composition cannot be faithfully replicated, the effect and function can be imitated. Human milk promotes growth of specific gut microorganisms, mainly of

Table 53.1 Confirmed prebiotics

Prebiotics	Food source
Galacto-oligosaccharides (GOS) Fructo-oligosaccharides (FOS) Inulin	Legumes, chickpeas, beans, and lentils Asparagus, garlic, and leeks Chicory, artichokes, onions, wheat, and bananas
Lactulose	Synthetic disaccharide

Table 53.2 New prebiotics

Prebiotics	Food source
Polyphenols	Cloves, dried herbs, green tea, and beans
Pectin	Cell wall components of fruit, asparagus, garlic, and leeks
Resistant starch	Potato, corn, and tapioca
Soy	Soybeans
Cellulose	Component of plant cell walls
β-Glucans	Soluble fibers found in oats and barley cereals
Mannose	Many fruits and vegetables
Xylo-oligosaccharides, arabinoxylo-oligosaccharides	Wheat bran
Maltose, malto-oligosaccharides	Products broken down from starch
Isomaltulose	Honey, sucrose, and sugarcane juice
Raffinose oligosaccharides	Beans, lentils, and peas

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the *Lactobacillus* and *Bifidobacterium* species, and they have also become the preferred target organisms when it comes to evaluating the effects of prebiotics [12].

The addition of two oligosaccharides to infant formulas based on cows' milk, galacto-oligosaccharides and inulin, has been shown to have a bifidogenic effect and to stimulate the growth of bifidi and lactobacilli. That is why they are added to infant formulas [13, 14] (Table 53.3).

The most abundant human milk oligosaccharide (HMO) in most mothers' breast milk is 2'-fucosyllactose (2'-FL). This molecule has recently been synthesized and shown to be structurally identical to the human milk. 2'-FL HMO is now available in some commercial infant formulas. Preclinical research has demonstrated that HMOs, and specifically 2'-FL, are more than just prebiotics. They have multiple functions, including immune stimulation, gut health, and cognition benefits [15–18].

Animal studies of *Il10* $^{-/-}$ mice have shown that post-weaning administration of specific oligosaccharides can shift the composition of the gut microbiota and reduce chronic inflammation. The expansion of *Ruminococcus gnavus* sets a positive microbial environment that lowers intestinal inflammation but at the cost of pro-inflammatory Gram-negative bacteria [19–21].

As our knowledge about the influence of prebiotics on the microbiota continues to grow, there have been continued discussions about the need to enlarge the definition. Recently, Bindels et al. proposed that a prebiotic should be defined as "a nondigestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host" [22]. Their proposed definition identifies the ingredient as the causative agent for changes in the microbiota. It also excludes the restrictive language related to selectivity and specificity while maintaining the need to identify a beneficial physiological effect. This helps pave the way for investigations into bacteria that have not been historically studied, such as bifidobacteria and lactobacilli. For example, butyrate-producing bacteria, such as *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, which is a mucin-degrading bacterium, have both been associated with beneficial health effects, including reduced inflammation and improved gut barrier function, respectively [6, 22].

More recently, new methods have been developed to investigate gut microbiota, such as next-generation sequencing. These methods have revealed more complex outcomes from prebiotic administration, with quite different and sometimes unexpected members of the microbiota being enriched by these supplements. In particular, several putatively beneficial autochthonous gut strains, including *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, have been shown to respond to specific prebiotics [23–28].

Consuming other substances, such as resistant starch and various soluble and insoluble fibers or human milk oligosaccharides, may also enrich other, as yet unidentified, strains of the gut microbiota. This may occur directly or via cross-feeding [29, 30]. However, there have been a lack of sequencing studies on the clinical application of quite a lot of the new prebiotics.

Taking into account these new observations, Bindels et al. have proposed enlarged definitions for prebiotics, as detailed above. So have Bird et al. [6, 22], who have further proposed that prebiotics should be viewed as "undigested dietary carbohydrates" that are fermented by colonic bacteria and create short-chain fatty acids as end products.

The current scientific definition of a prebiotic was reviewed by a panel of experts in microbiology, nutrition, and clinical research that was convened by the International Scientific Association for Probiotics and Prebiotics in 2016. The present consensus definition is "a substrate that is selectively utilized by host microorganisms conferring a health benefit." This notion is based on three inner parts: a substance, a physiological effect, and a mechanism [4, 31, 32] (Table 53.4).

Table 53.3 Prebiotics in infant formulas

Prebiotics	
Galacto-oligosaccharides (GOS)	GOS
Fructo-oligosaccharides (FOS)	FOS
2'-Fucosyllactose (2'-FL)	2' FL

Possible Effects of Prebiotics

If we are to understand how prebiotics work, and more importantly how to use them to influence the microbiota and improve health, we need to realize that the microorganisms that live in the gut are part of complex functional ecosystems.

Microbes play several roles within these ecosystems, including converting incoming dietary carbohydrates and degrading proteins into metabolites. These can then have a

Table 53.4 Prebiotic effects and outcomes

Effects	Outcomes
Immune modulation Improve bowel function Increase absorption of mineral	Reduce type 2 T helper responses, atopy Constipation, infantile colic Calcium=> improve bone health
Metabolic regulation	Favoring gluconeogenesis and lipid biosynthesis
Decrease protein fermentation	Reduce toxic metabolites
Provide nutrients for colonocytes	Stimulates proliferation of colonocytes

positive or negative effect upon the health of the host (Fig. 53.1) [33–35].

The prebiotics that are currently available are predominantly based on carbohydrates, but other substances might exert prebiotic effects, such as polyphenols and polyunsaturated fatty acids [32]. One example of a polyphenol is water-insoluble cocoa fraction. A gut model showed that this substantially increased bifidobacteria, lactobacilli, and butyrate production [36].

Carbohydrates with a low molecular weight are very well taken up by microorganisms such as bifidobacteria, which have a wide range of enzymes, namely, cell-associated and extracellular glycosidases. Bifidobacteria also have specific transport systems, allowing them to rapidly assimilate low-molecular-weight sugars [37].

Other bacteria, such as members of the *Bacteroides* genus, are adept at breaking down high-molecular-weight polysaccharides [38].

Some microorganisms might be regarded as keystone species, as they have the ability to initiate digestion of particular substrates [39]. One example is the *Ruminococcus* species, which can promote the degradation of resistant starch [40].

After they have been liberated, low-molecular-weight dextrins are then metabolized by the microbial community. The pathway from a polysaccharide to a short-chain fatty acid (SCFA) is, therefore, a complex and indirect network of metabolism. We know that acetate and lactate, which are the main metabolic end products of bifidobacteria and lactic acid bacteria, are also used by other microorganisms to produce, for example, propionate [41] and butyrate [42].

Some of the ecological networks that are involved in the metabolism of carbohydrates have been clarified [43, 44], but the extent to which they operate in the gut is not completely known at the present time (Fig. 53.1).

Immune Modulation

Some data have shown that those prebiotic interventions can reduce type 2 T helper cell responses and therefore affect allergies, although the precise mechanisms are uncertain.

The most helpful data have come from studies of infants. For example, GOS and long-chain FOS were administered in infant formula in a double-blind, randomized, placebo-controlled trial of 259 infants. The probiotic formula was associated with a reduced incidence of atopic dermatitis, wheezing, and urticaria, and the levels were more than 50% lower in this group than the controls who received a non-prebiotic formula [45–47].

Consuming prebiotics can improve immunity functions by increasing the population of protective microorganisms. Prebiotics can also induce the expression of immunity molecules, especially cytokines [48].

A prospective, double-blind, placebo-controlled trial, which has not been replicated yet, looked at healthy term-born infants at risk of atopy, who were fed with hypoal-

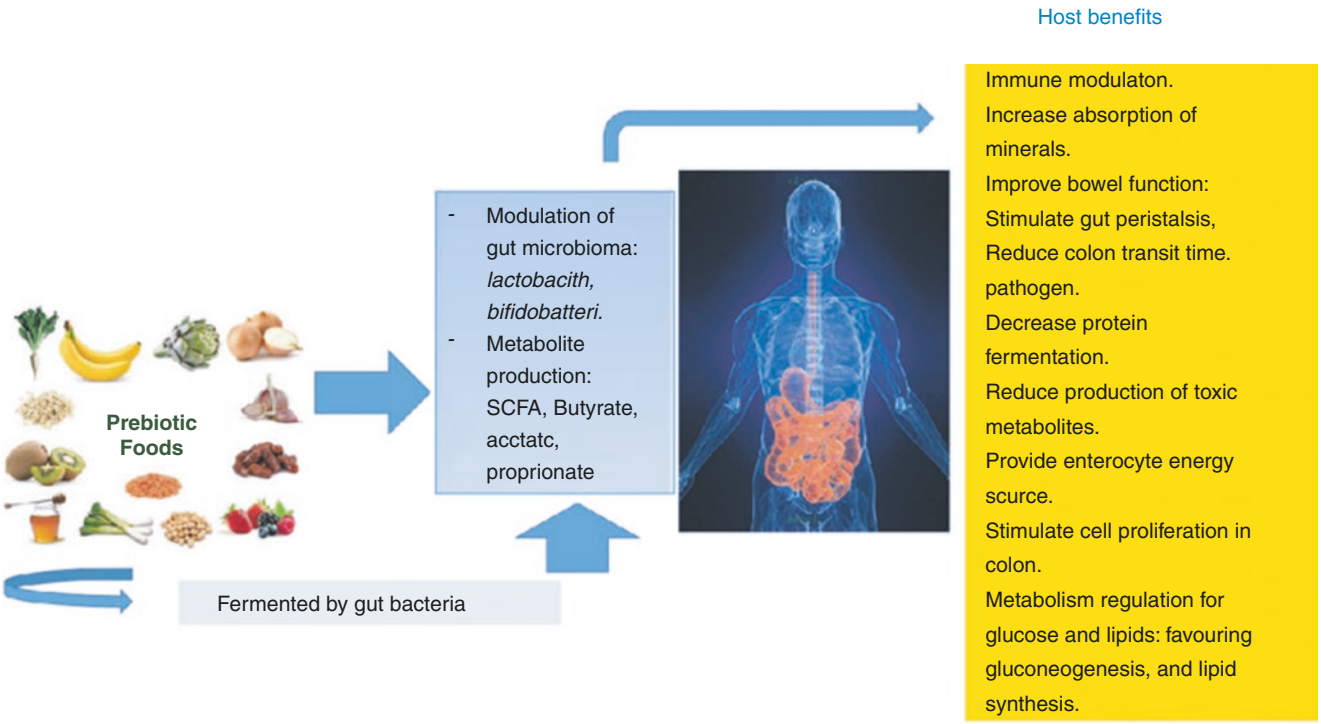

Fig. 53.1 Functions of prebiotics

lergenic formula supplemented with prebiotics for 6 months. The authors reported that they had a greater than fivefold reduction in the prevalence of allergies 5 years after they received the formula [49].

Improved Bowel Function

Improvements in bowel function have often been ascribed to simple fecal bulking, because individuals consume more dietary fiber [50]. However, animal studies have shown that the SCFAs produced by fermenting prebiotics can regulate gut hormones and that these, in turn, modulate the local motor responses of the gut [51].

The humectant water-binding capacity of prebiotic carbohydrates also soften stools, making their passage easier [52].

There are surprisingly few studies on the effect of prebiotics on bowel function, although those randomized trials that have been carried out have consistently reported improvements in stool consistency and the frequency of defecation [53, 54].

A systematic review and meta-analysis indicated that short-chain β-fructan supplements had a positive effect on bowel function, by significantly increasing the frequency of bowel movements and reducing constipation [55].

Increased Mineral Absorption

Minerals are mainly absorbed in active transport mechanisms in the small intestine, and increasing calcium could have a substantial, positive effect on bone mineral and good health. We know that when prebiotics are fermented this leads to the production of SCFAs, which reduces luminal pH. This can increase calcium solubility and, therefore, absorption [56]. One problem with proving that this process works is that many of the calcium salts in supplements and food have pH-dependent solubility and limited availability. Depending on the starting pH, the solubility of calcium can actually increase as the pH increases [57]. One study showed that when young adolescents consumed a mixture of FOS and inulin or GOS, this showed marked increases in the amount of calcium absorbed and mineralized in their bones [58].

Prebiotics in Infant Formulas

Prebiotics are commonly used to supplement infant formula. These include prebiotic oligosaccharides that stimulate *Bifidobacterium* growth and aim to mimic the high levels of these commensal bacteria in the guts of breastfed infants. Studies suggest that probiotic supplements may be beneficial in preventing and managing disease, by reducing the risk of necrotizing enterocolitis in preterm infants and treating minor digestive symptoms, such as constipation and infantile colic [15–21, 59–61].

Bourewicz et al. used Illumina HiSeq sequencing of polymerase chain reaction amplified 16S rRNA gene fragments to investigate the composition of fecal microbiota in infants aged 2–12 weeks who were receiving either breastmilk, infant formulas fortified with prebiotics, or mixed feeding. They showed that formulas supplemented with either GOS (0.24–0.50 g/100 ml) or GOS and FOS (9:1 ratio, total 0.6 g/100 ml) had a strong bifidogenic effect when compared to traditional formulas. They also resulted in altered patterns of microbial colonization within the infants' developing gastrointestinal tracts [62].

Possibly the strongest support for using prebiotics comes from prebiotic infant formulae. Such products are now routinely supplemented with mixtures of GOS and fructans [63, 64], and this 9:1 ratio blend of prebiotics has been shown to reduce respiratory tract infections to the levels found in breastfed infants [13, 65, 66]. There is less evidence that prebiotics with preclinical trial data, specifically those that have focused on 2'-FL, are more than just a prebiotic and have multiple functions, including immune, gut, and cognition benefits [15–18].

A Cochrane review [67] that examined the dietary treatment of infantile colic included a study that compared a partially hydrolyzed, lower-lactose, whey-based formula containing prebiotics, namely, oligosaccharide GOS and FOS, with a standard formula with simethicone. The trial comprised 267 infants, and it found that both groups experienced a decrease in colic episodes, which was a secondary outcome after seven days. The partially hydrolyzed formula fell from a mean of 5.99 ± 1.84 episodes to 2.47 episodes ±1.94, while the standard formula fell from 5.41 ± 1.88 episodes to 3.72 ± 1.98. After 2 weeks, the difference between the two groups was significant, with a mean of 1.76 ± 1.60 episodes for the partially hydrolyzed group and 3.32 ± 2.06 episodes for the standard formula. The authors confirmed there were no adverse effects in either group [60].

Conclusions

Prebiotics are substrates that are selectively used by host microorganisms and confer health benefits. They provide a defense against pathogens, as well as immune modulation; favor mineral absorption; and improve bowel function, metabolic effects, and satiety [68]. Pediatric researchers have shown a particular interest in their relationship with overall human health in recent years.

Our understanding of how prebiotic fibers influence the gastrointestinal microbiota, including those considered

prebiotics, is emerging. However, more research is needed to discover if modulating the composition and function of the human gastrointestinal microbiota leads to health benefits.

Larger prospective studies are needed to determine the associations between prebiotic supplements and disease. These should be well-controlled clinical studies, ideally randomized controlled trials, that study single ingredients and use crossover designs with washout periods. They should assess the impact of fiber on the gastrointestinal bacterial taxa and also focus on microbial metabolites and other physiological measures of health, such as body composition, blood cholesterol, glycemia, and gut inflammation. Where possible, the use of new methods to study gut microbiota will further strengthen these investigations.

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Probiotics in Pediatric Gastroenterology

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Introduction

A balanced gut microbiota is crucial for health. Conversely, dysbiosis, which refers to altered gut microbiota diversity and composition (i.e., changes at the level of phylum, genus, or species) [1], contributes to the development of gastrointestinal and extraintestinal diseases, such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), necrotizing enterocolitis (NEC), allergy, obesity, type 1 diabetes, and autism (in addition to other factors such as genes or environmental factors) [2, 3]. However, the exact microbiota changes remain a matter of debate. It also remains to be determined whether the gut microbiota alterations are a cause or a consequence of these disorders. For none of the diseases has a specific "microbiota signature" been identified. The lack of standardized methods for microbiota evaluation may be responsible for inconsistences among studies. However, efforts to standardize operating procedures designed to ensure the robustness of data in the microbiota field are being made [4]. Still, the association between a low diversity of gut microbiota, which may be considered a marker of dysbiosis, and disease has been documented in several studies. From a functional point of view, low gut microbiota diversity affects the gut-liver axis and is associated with the potential for reduced butyrate-producing bacteria, increased mucus degradation, reduced hydrogen and methane production combined with increased hydrogen sulfide formation, and increased hepatic oxidative stress, inflammation, and fibrogenesis [5, 6].

Targeting the gut microbiota with probiotics, prebiotics, synbiotics, postbiotics, and fecal microbiota transplants could potentially benefit human health and treat or reduce the risk of diseases. These interventions are currently gaining worldwide popularity and are increasingly being used in the pediatric population.

In this chapter, current evidence based on the findings from the latest meta-analyses of randomized controlled trials (RCTs) on the efficacy of probiotics in pediatric gastroenterology are summarized. If available, recent recommendations made by recognized scientific societies such as the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), the American Gastroenterology Association (AGA), and the European Crohn's and Colitis Organization (ECCO) are presented. To identify relevant data, searches of MEDLINE and the Cochrane Library databases were performed in July 2020 to locate publications preferentially published in English in the last 5 years.

Definition and Mechanismss of Probiotics

A consensus definition developed by the International Scientific Association for Probiotics and Prebiotics (ISAPP) defines probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [7]. Commonly used probiotics are bacteria from the genus *Lactobacillus* or *Bifidobacterium*, as well as a yeast, *Saccharomyces boulardii*. Each microorganism should be defined by its genus, species, and strain designations. Of note, the genus of *Lactobacillus* has been recently reclassified into 25 genera, which includes 23 novel genera [8]. For example, the new name for *Lactobacillus rhamnosus* is *Lacticaseibacillus rhamnosus*. However, the abbreviations of microorganisms remained the same (i.e., *L. rhamnosus*). Species names and strain designations did not change [8].

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Besides traditional probiotics, next-generation probiotics are currently under investigations, including *Roseburia* spp., *Akkermansia* spp., *Propionibacterium* spp., and *Faecalibacterium* spp., genetically modified organisms for delivering molecules in situ and live biotherapeutic products [9, 10].

As probiotics cover a range of living microorganisms, potential mechanisms of action differ. Possible main mechanisms include [11]:

- Production of metabolites such as short-chain fatty acids, the majority of which are acetate, propionate, and butyrate.
- Modulation of the composition and/or activity of the host microbiota (e.g., through colonization).
- Enhancement of epithelial barrier integrity.
- Modulation of the host immune system.
- Adherence to the mucosa and epithelium, with inhibition of pathogen adhesion and/or growth.
- Production of enzymes (e.g., lactase to promote lactose digestion).
- Production of bacteriocins.

Some of these mechanisms are shared by many microorganisms [7, 12]; however, some (e.g., immunological, production of specific bio-actives) are strain-specific mechanisms [7]. Considering the latter, when evaluating the health benefits and safety of probiotics, the focus should be on individual probiotic strains, not on probiotics in general. The effects of probiotics are strain- or combination-specific, not species-specific [13]. The efficacy is also disease-specific [14]. The clinical effects and safety of any single probiotic or combination of probiotics should not be extrapolated to other probiotics or conditions. Lastly, while some subjects respond to probiotic interventions, others do not [15]. Thus, it is likely that the response to probiotics is subject-specific.

For a guide on how to choose a probiotic, see Table 54.1. For a summary of the clinical effects of probiotics in children, see Table 54.2.

Many factors may contribute to the effects of probiotics, including diet, baseline microbiota, dose, matrix, manufacturing conditions, use of concomitant medications (e.g., gut microbiota modifiers such as antibiotics, proton pump inhibitors), and, in case of infectious diarrhea, etiology/vaccination status. Some recent studies have suggested, based on findings in animal and human experiments, that probiotic administration (at least the combination of 11 strains used by the investigators) does not consistently change the gut microbiota composition and that individual responses to probiotic administration differ. It was also suggested that probiotic effects, including the lack of an effect, may differ depending on the indigenous microbiota and gene expression profiles [16, 17].

Table 54.1 How to choose a probiotic (based on reference [9])

Genus, species, strain	Only probiotic strains for which identity and genetic stability have been demonstrated should be used Example of an identification: *Lacticaseibacillus* [formerly known as *Lactobacillus*] (genus) *L. rhamnosus* (species) GG (strain) ATCC 53103 (microbiological culture deposition)
Multi-strain vs. single-strain products	A multi-strain probiotic per se does not guarantee more benefit. For each product, the effect must be documented in well-designed RCTs
Expiration date	The manufacturer should guarantee: The *minimum* number of live cells throughout the shelf-life of the product The *maximum* number of live cells at the time of manufacturing (no guarantee on the viability by the end of shelf-life)
Storage needs	Storage needs recommended by the manufacturers must be followed
Dose	One dose cannot be assumed to be effective for all strains A larger dose is not always better It is prudent to use the treatment regimen proven to be effective in well-designed and executed RCTs for the same indication
Quality	Each formulation is subject to different regulatory processes and quality control processes
Evidence	See Table 54.2

ATCC American Type Culture Collection, *RCTs* randomized controlled trials

Management of Conditions with Probiotics

Treatment of Acute Gastroenteritis

The key treatment of acute gastroenteritis is oral rehydration with low osmolarity oral rehydration solution (ORS) [18, 19]. However, despite being effective, inexpensive, and widely available, ORS remains underused. Insufficient acceptability of ORS by caregivers and physicians may be due to the lack of an effect on the duration of diarrhea or stool output [19].

Until 2020, many, if not all, professional societies and group of experts advocated the use of probiotics with documented efficacy for the management of acute gastroenteritis [19–22]. Currently, the recommendations differ, possibly reflecting negative (null) studies questioning the efficacy of some strains with previous positive recommendations [23, 24].

In 2020, the ESPGHAN Working Group (WG) on Probiotics identified 16 systematic reviews and meta-analyses published since 2010, which included more than 150 RCTs. The WG made weak (also known as conditional) recommendations *for* (in descending order in terms of the number of trials evaluating any given strain) the following: *S.*

Table 54.2 Effects of probiotics in children. ESPGHAN and AGA recommendations

Condition	Society	Recommendation
Treatment of acute gastroenteritis	ESPGHAN 2020	Conditional (weak) recommendation *for*: *S. boulardii* (250–750 mg/day, for 5–7 days) (low to very low certainty of evidence) *L. rhamnosus* GG (ε10^{10} CFU/day, typically 5–7 day) (very low certainty of evidence) *L. reuteri* DSM 17938 (1×10^8 to 2×10^8 to 4×10^8 CFU/day, for 5 days) (low to very low certainty of evidence) *L. rhamnosus* 19070–2 and *L. reuteri* DSM 12246 (2×10^{10} CFU of each strain/d, for 5 days) (very low certainty of evidence) Strong recommendation *against*: *L. helveticus* R0052 and *L. rhamnosus* R0011 (moderate certainty of evidence) Weak recommendation *against*: *Bacillus clausii* O/C, SIN, N/R, and T (very low certainty of evidence)
	AGA 2020	Against the use of probiotics in children with acute infectious gastroenteritis in North America (conditional recommendation, moderate quality of evidence)
Prevention of AAD	ESPGHAN 2016	Strong recommendation *for*: *L. rhamnosus* GG (moderate quality of evidence) *S. boulardii* (moderate quality of evidence)
	AGA 2020	Not addressed
Prevention of *C. difficile* diarrhea	ESPGHAN 2016	*S. boulardii* (moderate quality of evidence)
	AGA 2020	*S. boulardii* A two-strain combination of *L. acidophilus* CL 1285 *and L. casei* LBC80R A three-strain combination of *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *B. bifidum*[a] A four-strain combination of *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. bifidum*, and *Streptococcus salivarius* subsp. *thermophilus*[a]
Prevention of NEC	ESPGHAN 2020	Conditional recommendation *for*: *L. rhamnosus* GG ATCC53103 (at a dose ranging from 1×10^9 CFU to 6×10^9 CFU) (low certainty of evidence) *B. infantis* Bb-02, *B. lactis* Bb-12, and *Str. thermophilus* TH-4 at 3.0 to 3.5×10^8 CFU (of each strain) (low certainty of evidence) No recommendation *for* or *against*: *L. reuteri* DSM 17938 (very low certainty of evidence) *B. bifidum NCDO 1453 and L. acidophilus NCDO 1748* (very low certainty of evidence) Conditional recommendation *against*: *B. breve BBG-001* *S. boulardii*
	AGA 2020	Combination of *Lactobacillus* spp. and *Bifidobacterium* spp.: *L. rhamnosus* ATCC 53103 and *B. longum* subsp. infantis[a] *L. casei* and *B. breve*[a] *L. rhamnosus*, *L. acidophilus*, *L. casei*, *B. longum* subsp. *infantis*, *B. bifidum*, and *B. longum* subsp. *longum*[a] *L. acidophilus* and *B. longum* subsp. *infantis*[a] *L. acidophilus* and *B. bifidum*[a] *L. rhamnosus* ATCC 53103 and *B. longum* Reuter ATCC BAA-999 *L. acidophilus*, *B. bifidum*, *B. animalis* subsp. *lactis*, and *B. longum* subsp. *longum*[a] *B. animalis* subsp. *lactis* (including DSM 15954) *L. reuteri* (DSM 17938 or ATCC 55730) *L. rhamnosus* (ATCC 53103 or ATCA07FA or LCR 35)
H. pylori infection	ESPGHAN and NASPGHAN 2017	Not recommended
Crohn's disease	ESPGHAN 2018	Not recommended
	AGA 2020	*Against* the use of probiotics, unless in the context of a clinical trial

(continued)

Table 54.2 (continued)

Condition	Society	Recommendation
Ulcerative colitis	ESPGHAN and ECCO 2018	A mixture of eight strains[b] or *Escherichia coli* Nissle 1917
	AGA 2020	*Against* the use of probiotics, unless in the context of a clinical trial
Pouchitis	ESPGHAN 2018	A mixture of eight strains[b]
	AGA 2020	A mixture of eight strains[b]
Functional abdominal pain disorders, including IBS	ESPGHAN or NASPGHAN	No addressed
	AGA 2020	IBS. Only in the context of a clinical trial
Infantile colic	ESPGHAN or NASPGHAN or AGA	Not addressed
Functional constipation	ESPGHAN and NASPGHAN 2014	Not recommended

AAD antibiotic-associated diarrhea, *AGA* American Gastroenterology Association, *CFU* colony-forming units, *ECCO* European Crohn's and Colitis Organization, *ESPGHAN* European Society for Paediatric Gastroenterology, Hepatology and Nutrition, *NASPGHAN* North American Society for Pediatric Gastroenterology, Hepatology and Nutrition, *NEC* necrotizing enterocolitis

[a]No strain specification was given for any of the strains

[b]*L. paracasei* DSM 24733, *L. plantarum* DSM 24730, *L. acidophilus* DSM 24735, *L. delbrueckii* subspecies *bulgaricus* DSM 24734, *B. longum* DSM 24736, *B. infantis* DSM 24737, *B. breve* DSM 24732, and *Str. thermophilus* DSM 247]

boulardii (low to very low certainty of evidence), *L. rhamnosus GG* (very low certainty of evidence), *L. reuteri* DSM 17938 (low to very low certainty of evidence), *L. rhamnosus* 19070-2, and *L. reuteri* DSM 12246 (very low certainty of evidence). The WG made a strong recommendation *against L. helveticus* R0052 and *L. rhamnosus* R0011 (moderate certainty of evidence) and a weak (conditional) recommendation *against Bacillus clausii* strains O/C, SIN, N/R, and T (very low certainty of evidence) [25].

In contrast, also in 2020, the AGA, based on the evaluation of 89 trials, made a conditional recommendation *against* the use of probiotics in children from North America with acute infectious gastroenteritis (moderate quality of evidence) [13]. The rationale for the negative AGA recommendation was that the majority of the studies were performed outside North America. Moreover, two large, high-quality null trials, performed in Canada and the USA, questioned the efficacy of probiotics, or more specifically the probiotics evaluated in these studies, for the management of children with acute gastroenteritis [23, 26].

Of note, the interpretation of a conditional recommendation *for* and a conditional recommendation *against* is similar. For clinicians, it means that different choices will be appropriate for different people. Clinicians should help each patient make decisions consistent with the patient's preferences. For patients, it means that the majority of individuals in this situation would want the suggested course of action, but many would not [27].

Prevention of Antibiotic-Associated Diarrhea

One of the potential mechanisms of antibiotic-associated diarrhea (AAD) is a direct effect of the antibiotics on the intestinal mucosa, resulting in alterations in the gut microbiota composition and the overgrowth of pathogens. *Clostridioides*, formerly known as *Clostridium difficile* (*C. difficile*), is the most common infectious cause of AAD [28]. However, other pathogens such as *Staphylococcus*, *Candida*, *Enterobacteriaceae*, and *Klebsiella* may be involved [29]. Other mechanisms include a decrease in the metabolism of the non-digested carbohydrates and primary bile acids and the reduced production of short-chain fatty acids [29]. The clinical manifestations of AAD range from mild diarrhea to colitis or fulminant pseudomembranous colitis.

Evidence from several meta-analyses has consistently shown that most of the tested probiotics significantly reduce the risk of AAD, including a 2019 Cochrane review [30]. The latter identified 33 RCTs involving 6352 participants and indicated a statistically significant reduction in the incidence of AAD in the probiotics groups compared with the control groups (8% vs. 19%, respectively, relative risk 0.45, 95% confidence interval 0.36 to 0.56), with a number needed to treat of 9 (95% CI 7 to 13). Single-strain meta-analyses found that, compared with placebo or no intervention, probiotics such as *S. boulardii* [31] or *L. rhamnosus* GG [32] reduced the risk of AAD.

For preventing AAD, in 2016, the ESPGHAN WG on Probiotics recommended using *L. rhamnosus* GG (moderate quality of evidence, strong recommendation) or *S. boulardii* (moderate quality of evidence, strong recommendation). Other strains or combinations of strains have been tested, but sufficient evidence is still lacking. If the use of probiotics for preventing *C. difficile*-associated diarrhea is considered, the ESPGHAN WG suggested using *S. boulardii* (low quality of evidence, conditional recommendation) [33].

In contrast, the AGA (2020) did not formulate any recommendations with regard to the use of probiotics for preventing AAD. However, the AGA conditionally recommended (based on low quality of evidence) certain probiotics for the prevention of *C. difficile* infection in children receiving antibiotic treatment. These include *S. boulardii*; or the two-strain combination of *L. acidophilus* CL 1285 *and L. casei* LBC80R; or the three-strain combination of *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *B. bifidum*; or the four-strain combination of *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B bifidum*, and *Streptococcus salivarius* subsp. *thermophilus* [13]. No strain specification was given for the

three-strain and four-strain combinations which may contribute to confusion for implementation of these recommendations.

Prevention of Necrotizing Enterocolitis

NEC is one of the most severe and life-threatening gastrointestinal diseases to occur in preterm infants, particularly those with a birth weight <1000 g. The factors involved in the pathogenesis of NEC include formula feeding rather than breastfeeding, intestinal hypoxia–ischemia, and colonization of the gut with pathogenic microbiota [34]. A number of previous meta-analyses consistently showed that the administration of probiotics to infants was associated with a reduced risk for NEC or death [35–37]. In 2020, both ESPGHAN [38] and AGA [13] published their recommendations on the use of probiotics for preventing NEC. While both were based on pair-wise systematic reviews and network meta-analyses [39], their conclusions differ. The only probiotic strain that was recommended by both societies is *L. rhamnosus* GG ATCC 53103. With regard to *L. reuteri* DSM 17938, the ESPGHAN did not formulate a recommendation for or against it, while the AGA conditionally recommends it. For details and specific recommendations from both societies, see Table 54.3.

Table 54.3 Probiotics for preventing NEC. Comparison of ESPGHAN and AGA recommendations

	ESPGHAN 2020	AGA 2020
Target population	Preterm infants	Preterm infants (less than 37 weeks GA), low-birth-weight infants
Intervention	Probiotics	Probiotics
Comparison	Placebo/no probiotics	Placebo/no probiotics
Outcomes	NEC, sepsis, and all-cause mortality	NEC, sepsis, and all-cause mortality
Recommendations formulated based on system atic reviews	Probiotic strain-specific systematic review and NMA	Probiotic strain-specific systematic review and NMA
Number of RCTs evaluated	51 RCTs, *n* = 11,231	63 RCTs, *n* = 15,712
Members of the guideline development group	Experts in the fields of neonatology, pediatric gastroenterology, and nutrition	Guideline panel: gastroenterologists Technical report: methodologists
Target audience	Not specified	North America
General recommendation	Only strain-specific recommendations were formulated	We suggest using a combination of *Lactobacillus* spp. and *Bifidobacterium* spp. for the prevention of NEC over no and other probiotics
Recommendations *for*	*L. rhamnosus* GG ATCC 53103	*L. rhamnosus* (ATCC 53103 or ATC A07FA or LCR 35)
	B. infantis Bb-02 + *B. lactis* Bb-12 + *Str. thermophilus*	*L. rhamnosus* ATCC 53103 and *B. longum* subsp. *infantis*
		L. casei and *B. breve*
		L. rhamnosus, *L. acidophilus*, *L. casei*, *B. longum* subsp. *infantis*, *B. bifidum*, and *B. longum* subsp. *longum*
		L. acidophilus and *B. longum* subsp. *infantis*
		L. acidophilus and *B. bifidum*
		L. rhamnosus ATCC 53103 and *B. longum* Reuter ATCC BAA-999
		L. acidophilus, *B. bifidum*, *B. animalis* subsp. *lactis*, and *B. longum* subsp. *longum*)
		B. animalis subsp. *lactis* (including DSM 15954)
		L. reuteri (DSM 17938 or ATCC 55730)
		L. rhamnosus (ATCC 53103 or ATC A07FA or LCR 35)

(continued)

Helicobacter pylori Infection

Unsatisfactory *H. pylori* eradication rates and therapy-associated side effects remain a problem. A number of systematic reviews and meta-analyses, focusing mainly on adults, have shown that probiotic supplementation improves eradication rates and/or reduces side effects of anti-*H. pylori* treatment [40, 41]. For pediatric patients, a 2017 systematic review and a network meta-analysis involving 29 trials (17 probiotic regimens, 3122 participants) showed that compared with the control groups, children in the probiotic groups experienced an increased eradication rate and reduced the risk of total side effects associated with *H. pylori* eradication therapy [42]. A single-strain meta-analyses found that compared with placebo or no intervention, *S. boulardii* given along with standard triple therapy significantly reduced the risk of overall *H. pylori* therapy-related adverse effects and increased the eradication rate [43]. However, data in children for *S. boulardii* were limited [44]. While in both analyses the addition of probiotics to standard triple therapy significantly increased the eradication rate, it was still below the desired level (≥90%) of success.

According to 2017 ESPGHAN/NASPGHAN *H. pylori* guidelines [45], the routine addition of either single or combination probiotics to eradication therapy to reduce side

Table 54.3 (continued)

	ESPGHAN 2020	AGA 2020
No recommendation *for* or *against*	*L. reuteri* DSM 17938 *B. bifidum* NCDO1453 and *L. acidophilus* NCDO 1748	
Recommendations *against*	*B. breve* BBG-001 *S. boulardii*	

GA gestational age, *NEC* necrotizing enterocolitis, *NMA* network meta-analysis, *RCTs* randomized controlled trials

effects and/or improve eradication rates is currently not recommended. This is in contrast to the recommendations in adults [46].

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) consists mainly of two distinct disorders: Crohn's disease and ulcerative colitis. A recent systematic review concluded that there is increasing evidence for differences in abundances of some bacteria in patients with IBD compared with controls [47], contributing to the initiation and maintenance of inflammation. However, the findings were not consistent, at least partially due to different methods used to analyze the microbiota.

A 2020 Cochrane review concluded that low certainty of evidence suggests that probiotics may induce clinical remission in patients with active ulcerative colitis when compared to placebo [48]. However, specific strain(s) were not identified. Another 2020 Cochrane review concluded that evidence is vague with regard to the efficacy or safety of probiotics, when compared with placebo, for induction of remission in patients with Crohn's disease [49].

In line with 2018 evidence-based guidelines by the ECCO and ESPGHAN, a mixture of eight strains [*L. paracasei* DSM 24733, *L. plantarum* DSM 24730, *L. acidophilus* DSM 24735, *L. delbrueckii* subspecies *bulgaricus* DSM 24734, *B. longum* DSM 24736, *B. infantis* DSM 24737, *B. breve* DSM 24732, and *Str. thermophilus* DSM 24731] or *Escherichia coli* Nissle 1917 may be considered as an effective treatment for maintenance in patients with mild ulcerative colitis as an adjuvant therapy or in those intolerant to 5-ASA; however, this recommendation is based on limited evidence [50].

In line with 2018 guidelines of the ESPGHAN Porto IBD Group [51], there is limited evidence in favor of using the mixture of eight strains (as above) or *L. reuteri* ATCC 55730 as an adjuvant to standard therapy for induction of remission in mild-to-moderate pediatric ulcerative colitis. The mixture of eight strains has also shown efficacy for maintaining remission and possibly preventing pouchitis in adults [48], but data in children are lacking [51].

For Crohn's disease, according to the same 2018 ESPGHAN guidelines, there is not enough evidence to suggest that probiotics are beneficial for the induction or maintenance of remission of Crohn's disease in children [51].

The AGA (2020), both in patients with ulcerative colitis and Crohn's disease, recommends *against* the use of probiotics, unless in the context of a clinical trial [13]. However, in adults and children with pouchitis, the AGA conditionally recommends the use of the eight-strain combination [*L. paracasei* subsp. *paracasei* DSM 24733, *L. plantarum* DSM 24730, *L. acidophilus* DSM 24735, *L. delbrueckii* subsp. *bulgaricus* DSM 24734, *B. longum* subsp. *longum* DSM 24736, *B. breve* DSM 24732, *B. longum* subsp. *infantis* DSM 24737, and *S. salivarius* subsp. *thermophilus* DSM 24731] over no or other probiotics.

Functional Gastrointestinal Disorders

Functional gastrointestinal disorders (FGDs) account for a substantial number of referrals to gastroenterology clinics. Management remains difficult, prompting interest in new and safe treatment options.

Treating Infantile Colic

Evidence suggests that the gut microbiota in subjects with infantile colic differs from that in an unaffected population, and first-pass meconium is associated with subsequent infantile colic [52]. In colicky infants, dysbiosis may affect gut motor function and gas production, leading to abdominal pain/colic [53].

A 2018 individual participant data meta-analysis, which included data from four RCTs involving 345 infants with colic, documented that the administration of *L. reuteri* DSM 17938 at a dose 1×10^8 colony-forming units (CFU) is likely to reduce crying and/or fussing time in breastfed infants with infantile colic, but its role in formula-fed infants is less clear [54]. Other meta-analyses have confirmed these findings [55, 56]. Whether the type of feeding matters is uncertain, as the incidence of infantile colic is similar among breastfed and formula-fed infants [57]. Data on other probiotics, either positive or negative, are too limited to allow one to draw reliable conclusions [58–61].

Preventing Infantile Colic

A 2019 Cochrane review identified six RCTs (involving 1886 infants) which compared probiotics with placebo for preventing infantile colic [62]. The pooled results of three RCTs in which LGG and two multi-strain products (one included four strains of lactobacilla and three strains of bifidobacteria and *Str. thermophilus* DSM 24731 and another included *L. rhamnosus* GG, LC705, *B. breve* Bb99, and *Propionibacterium freudenreichii* ssp. *shermanii*) were

assessed found a similar occurrence of new cases of colic in the probiotics and placebo groups. The pooled results of three other RCTs found, in the probiotics group compared with the placebo group, reduced duration in crying time at study end (MD −32.6 min/day, 95% CI −55.6 to −9.5). However, one of the included studies evaluated a prebiotic formula with added probiotic strains; thus, this was a synbiotic intervention. At the strain level, the effect was particularly evident for *L. reuteri* DSM 17938 administered at a dose of 1×10^8 CFU to newborns each day for 90 days [63]. Other probiotics were also studied; however, evidence is limited [64].

Functional Abdominal Pain Disorders

Functional abdominal pain disorders (FAPDs), particularly IBS, are evidently associated with gut microbiota dysbiosis [65]. However, no clear microbiota signature has been identified. It also remains to be determined whether the gut microbiota alterations are a cause or a consequence of the disease. As most data have been obtained in adults [65], it remains unclear whether the findings can be extrapolated to children.

A 2018 systematic review concluded that there is insufficient evidence for the use of probiotics in children with FAPDs [66]. Only *L. rhamnosus* GG (three RCTs) reduced the frequency and intensity of abdominal pain in children with IBS. In children with IBS, a multicenter, crossover RCT using a mixture of eight probiotic strains (*Str. thermophilus* BT01, *B. breve* BB02, *B. longum* BL03, *B. infantis* BI04, *L. acidophilus* BA05, *L. plantarum* BP06, *L. paracasei* BP07, *L. delbrueckii* subsp. *bulgaricus* BD08) was found to be safe and more effective than placebo in ameliorating symptoms and improving quality of life [67]. Evidence on *L. reuteri* DSM 17938 (five RCTs using different methods of pain assessment) for treating FAPDs is inconsistent. Compared with placebo, *L. reuteri* DSM 17938 improved abdominal pain in three RCTs [68–70], reduced functional disability but not abdominal pain in one RCT [71], and was no better than placebo in one trial [72]. Mixtures of *B. infantis*, *B. breve*, and *B. longum* (one RCT) or *B. lactis* (one RCT) were not effective in children with FAPDs [66].

There are no specific recommendations from ESPGHAN or NASPGHAN. The AGA 2020 guidelines noted with regard to IBS that there are many studies; however, significant heterogeneity in study design, outcomes, and probiotics used resulted in no recommendations for the use of probiotics in symptomatic children and adults with IBS (except in the context of a clinical trial).

Functional Constipation

Functional constipation is a frustrating symptom affecting 3% of children worldwide. Treatment is often difficult and long-lasting. Moreover, more than 30% of the children dislike the taste of the conventional laxatives available. Some studies suggest differences in gut microbiota in patients with versus without functional constipation. However, no firm conclusions can be made due to inconsistent findings and methods used in studies [73].

A 2017 systematic review of RCTs identified seven RCTs with a total of 515 participants performed in children with functional constipation [74]. Pooled results of two RCTs showed no significant difference between the *L. rhamnosus casei* Lcr35 and placebo groups with respect to treatment success. Other probiotics studied in single trials only included *L. rhamnosus* GG; *L. reuteri* DSM 17938; *B. lactis* DN-173 010 [and yogurt starter cultures: *L. delbrueckii* ssp. *bulgaricus* (CNCM I-1632 and I-1519), *Str. thermophilus* CNCM I-1630, and *Lactococcus cremoris* (CNCM I-1631)]; *B. longum* [and yogurt starters, *L delbrueckii* subspecies *bulgaricus* and *Str. thermophilus* from the YF-L812 commercial culture]; and a mixture of seven strains (*L. casei* PXN 37, *L. rhamnosus* PXN 54, *Str. thermophilus* PXN 66, *B. breve* PXN 25, *L. acidophilus* PXN 35, *B. infantis* PXN 27, and *L. bulgaricus* PXN 39). There was no significant difference between the probiotic and control groups with respect to treatment success [74]. Similar conclusions were reached by the authors of a 2018 systematic review [66]. The findings of both systematic reviews support current ESPGHAN/NASPGHAN recommendations that probiotics should not be used in the treatment of functional constipation in children [75].

Cystic Fibrosis

Cystic fibrosis (CF) is a common, severe, autosomal recessive genetic disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. There is evidence from metagenomics and metabolomics studies that the CFTR gene mutation contributes to gut dysbiosis [76]. Frequent use of antibiotics is an additional risk factor contributing to dysbiosis in children with CF.

A 2020 Cochrane review [77] of probiotics for people with CF included 12 RCTs (among them, 8 RCTs included children only). Probiotics (as a group) might have some limited health benefits. Compared with placebo, there was reduced fecal calprotectin in the probiotic groups. For clinical outcomes, there were a reduced number of pulmonary exacerbations in those receiving probiotics compared with placebo after 12 months, albeit this difference was of borderline significance. There was no strong effect of probiotic treatment on weight, lung function, or hospitalizations. Similar conclusions were reached by the authors of earlier systematic reviews focusing on children only [78, 79]. However, so far, CF guidelines have not been updated to recommend the use of any of the studied probiotics in patients

with CF. Among others, specific probiotic strains, doses, and durations of treatment need to be established.

Other Diseases

A number of RCTs have evaluated various probiotics for preventing or treating other diseases. Both positive and negative (null) studies have been published. With few exceptions (e.g., pancreatitis), for most of these diseases, explicit *for* or *against* recommendations have not been formulated. Different choices may be appropriate for different patients. Clinicians should help each patient make decisions consistent with their preferences. Among others, the other conditions for which probiotics have been studied include:

- Celiac disease (e.g., *B. infantis* NLS in adults [80], *B. longum* CECT 7347 in children [81], *B. breve* BR03 and *B. breve* B632 [82, 83].
- Non-celiac gluten sensitivity (e.g., *B. longum* ES1 [84].
- Type 1 diabetes (e.g., *L. rhamnosus* GG and *B. lactis* Bb12) [85].
- Small bowel bacteria overgrowth (inconsistent results) [86, 87].
- Pancreatitis (in adults, a multispecies probiotic preparation increased mortality from mesenteric ischemia) [88]; the use of probiotics is not recommended).
- Non-alcoholic fatty liver disease (e.g., *L. rhamnosus* GG [89]; a mixture of *L. acidophilus* ATCC B3208, *B. lactis* DSMZ 32269, and *B. bifidum* ATCC SD6576; *L. rhamnosus* DSMZ 21690 [90]; a mix of eight strains (*Streptococcus thermophilus*, bifidobacteria [*B. breve*, *B. infantis*, *B. longum*], *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, and *L. delbrueckii* subsp. *bulgaricus*)).
- Autism spectrum disorders (e.g., *L. plantarum* PS128 [91].
- Caries (e.g., *L. rhamnosus* GG [92].
- Eczema (various *Lactobacillus* and *Bifidobacteria* species [93].

Safety of Probiotics

A 2018 systematic review found that more than a third of the 384 trials evaluated did not provide information on harms, and only 2% adequately reported adverse events [94]. While uncertainty remains due to a lack and/or inadequate reporting of harms, overall, probiotics are considered safe for use in otherwise healthy populations. Caution should be exercised with regard to all probiotics, including *S. boulardii* [95] and *L. rhamnosus* GG [96], in specific patient groups. Risk factors for adverse events include immunosuppression, prematurity, critical illness, presence of structural heart disease, hospitalization, presence of a central venous catheter, and the potential for translocation of probiotics across the bowel wall [97, 98]. The effects of long-term administration of probiotics remain largely unknown. If probiotics are to be given early in life, for a prolonged time, safety and quality issues need special attention.

Quality of Probiotics

There is also a concern with regard to the quality of products targeting the microbiota, probiotics in particular. This issue was recently reviewed by the ESPGHAN Working Group on Probiotics [99]. The Working Group suggested a more stringent quality control process to ensure "that the probiotic content as mentioned on the label meets the actual content throughout the shelf life of the product, while no contamination is present." Probiotic product quality through an independent third party would be desirable [100]. However, at present, there is no general standardization of regulatory framework, and it may differ between countries.

Conclusions

Interventions modifying the gut have the potential to prevent gastrointestinal and extraintestinal diseases. Despite the evidence to support the use of specific interventions in some clinical situations, further studies confirming the effect(s) and defining the type, dose, and timing of probiotics are still often required. As not all interventions within the group of probiotics are equal, the efficacy as well as safety should be established individually for each specific intervention. The use of interventions targeting the gut microbiota with no documented health benefits should be discouraged.

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Postbiotics

55

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Introduction

Interest in gut microbiota for health regulation has rapidly increased. Understanding their mechanisms, effects, and regulation is important for developments in their application, as a balanced microbiome is related to health, while an imbalanced microbiome or dysbiosis is related to many health problems. These microbiota are found within the gastrointestinal tract, but they have a wider impact. Therefore, the development of microbiota-modifying products, both foods, supplements, and pharmaceuticals, has increased. In this context, knowledge of the biotic family is of importance. Previously, the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus panel defined three members of this family: probiotics, prebiotics, and synbiotics [1–3]. Recently, the family was completed with postbiotics [4, 5]. Descriptions of the consensus terms are presented in Fig. 55.1.

In this chapter, recent developments on postbiotics are presented, and current evidence on the efficacy and safety of postbiotics for pediatric population is summarized. To obtain evidence on the health benefits of postbiotics in children, the Cochrane Central Register of Controlled Trials and MEDLINE databases were searched in July 2020 for randomized controlled trials (RCTs) or meta-analyses that compared postbiotics with placebo or no therapy.

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Definition and Mechanisms of Action of Postbiotics

The concept that non-living microorganisms could promote or preserve health is not new, and several terms have been used to describe such substances (e.g., paraprobiotics, parapsychobiotics, ghost probiotics, or metabiotics). However, in recent years, the term "postbiotic" has been used most often. A consensus definition was established by ISAPP to enable the use of a single, well-defined, and widely understood term rather than different terms. According to this definition, a postbiotic is "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the target host" [4]. Effective postbiotics must contain inactivated microbial cells with or without metabolites or cell components that contribute to observed health benefits. Purified compounds synthesized by microorganisms, such as antibiotics, are not considered postbiotics. Compared with probiotics, postbiotics offer several advantages. First, they are safe. Postbiotics can be reasonably expected to have a better safety profile than probiotics because they have lost the capacity to replicate and thus cannot cause bacteremia, a risk (albeit extremely rare) of probiotic administration. Still, postbiotics cannot be presumed to be safe based solely on the safety profile of the progenitor microorganism, and a safety assessment of any postbiotic is needed prior to use. Second, postbiotics have inherent stability, both during industrial processes and storage. An example of the safety assessment of a potential probiotic, *Bacteroides xylanisolvens*, was reported by the European Food Safety Authority [6, 7]. Maintaining the stability of live microorganisms is a technological challenge, but inanimate microorganisms can readily be used to create products with a long shelf life. Postbiotics may also be more suited than probiotics to geographic regions that do not have reliable cold chains or whose ambient temperature may cause problems for the storage of live microorganisms [4, 5].

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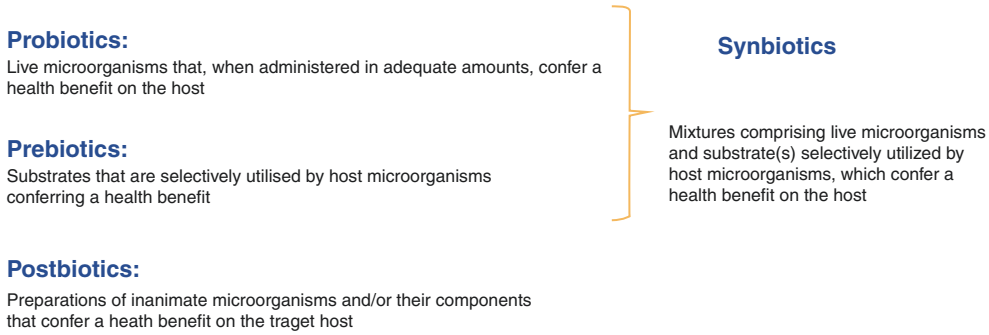

Fig. 55.1 Definitions of the members of the Biotic family: probiotics, prebiotics, synbiotics and postbiotics

The mechanisms of action for postbiotics remain unclear. Moreover, the potential mechanisms of action differ, as postbiotics derive from a range of living microorganisms. Recently, ISAPP experts proposed five main mechanisms of postbiotics, as discussed in detail elsewhere. These mechanisms include modulation of the resident microbiota, enhancement of epithelial barrier functions, modulation of local and systemic immune responses [4], modulation of systemic metabolic responses, and systemic signaling via the nervous system [4, 5].

The Science of Inactivated Probiotics and Other Microorganisms

Although probiotics are defined as live when administered, all probiotic preparations include dead and injured microorganisms. The potential impact of these non-viable bacterial cells and their components on probiotic functionality has attracted some attention, but it has been mostly neglected [5]. Fermented foods, such as yogurt, may contain a significant number of non-viable microbial cells, particularly after prolonged storage but also after processing with methods such as pasteurization or baking (e.g., sourdough bread).

Fermented foods have long been a significant part of the human diet and nutrition. Fermentation has a significant impact on the physical properties and potential health effects of many foods, especially milk and plant-based foods. It is most often mediated by lactic acid bacteria, and the results produce a range of cellular structures and metabolites that are associated with human health, including various cell surface components (inactivated artificially or naturally); short-chain fatty acids (SCFAs), such as lactic and acetic acids; and other metabolites. SCFAs have a number of roles in human health, including fighting pathogens and nurturing intestinal epithelial cells. The effector molecules of microorganisms in fermented food have been considered to be similar to those of probiotics, but fermentation products may have different effects.

Bacterial lysates of common bacterial respiratory pathogens have been used for decades to prevent pediatric respiratory diseases via general immune-stimulating mechanisms, which have been postulated but are not yet well understood.

Postbiotics can be based on current inactivated probiotics or novel microorganisms. Such organisms include, for example, *Akkermansia muciniphila*, *Eubacterium hallii*, *Streptococcus*, and *Faecalibacterium* species as well as some fructophilic lactic acid bacteria. Certain yeasts have also been reported as potential sources of postbiotics [5, 6, 8, 9]. To date, only one larger study in adults has been conducted with non-viable *B. bifidum* HI-MIMBb75 cells to examine their potential to alleviate irritable bowel syndrome (IBS) and its symptoms [10]. Further studies need to be conducted to confirm the results.

Postbiotics in Children

Fermented Formulas

Fermented formulas (i.e., those fermented with lactic acid-producing bacteria during the production process and not containing significant amounts of viable bacteria in the final product) are available in some countries. In 2007, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition reviewed evidence on fermented infant formulas published until April 2006. As only two randomized controlled trials (RCTs), which included 933 infants, were identified, the committee concluded that no conclusions could be drawn regarding the use and effects of fermented formulas for infants [11]. A 2015 systematic review updated this review with new evidence [12]. Five RCTs, which evaluated fermented infant formulas containing *Bifidobacterium breve* C50 and *Streptococcus thermophilus* with a sample of 1326 infants, met the inclusion criteria. Overall, the limited available evidence suggested that the use of fermented milk formula does not offer clear additional benefits compared to standard infant formula. At the same time, no negative health effects have been documented. A more recent study evaluated the effects of a newly developed fermented infant formula combining short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) with

Lactofidus™. The 50% fermented formula with scGOS/lcFOS was well tolerated and associated with lower overall crying time, a lower incidence of infantile colic, and a stool-softening effect in healthy term infants. However, as fermented formula was supplemented with scGOS/lcFOS, it is unclear which modification (i.e., the fermented formula or prebiotic oligosaccharides) was responsible for the effects [13].

Data on the use of fermented formulas in preterm infants are limited to one RCT, which evaluated the effect of a formula fermented by *B. breve* and *Str. thermophilus*. In infants fed fermented preterm formula, compared with standard preterm formula, there was a reduced incidence of abdominal distension as well as significantly lower fecal calprotectin levels [14].

Prevention and Treatment of Common Infectious Diseases

A 2020 systematic review [15] (search date: March 2019) evaluated evidence on the use of postbiotics to prevent and treat common infectious diseases among children younger than 5 years. Seven RCTs involving a total of 1740 children met the inclusion criteria. In therapeutic trials, compared with the placebo, supplementation with heat-killed *Lactobacillus acidophilus* LB reduced the duration of diarrhea (four RCTs; $n = 224$; mean difference [MD], −20.31 h; 95% CI −27.06 to −13.57). For preventive trials, the pooled results from two RCTs ($n = 537$) showed that, compared with the placebo, heat-inactivated *L. paracasei* CBA L74 reduced the risk of diarrhea (relative risk [RR] 0.51; 95% CI 0.37–0.71), pharyngitis (RR 0.31; 95% CI 0.12–0.83), and laryngitis (RR 0.44; 95% CI 0.29–0.67). The authors concluded that there is only limited evidence to recommend the use of specific postbiotics for treating pediatric diarrhea and preventing common infectious diseases among children. Further trials are needed to evaluate the effects of different postbiotics.

Two additional studies were identified. The first RCT investigated the effect of micronutrients (including zinc) with or without heat-inactivated *L. acidophilus* compared to a placebo in 75 infants aged 6–12 months who are at high risk for diarrhea-related mortality (defined as at least one episode of diarrhea in the preceding 2 weeks). The prevalence of diarrhea was 26% in the micronutrient with *L. acidophilus* group, 15% in the micronutrient group, and 26% in the placebo group. The difference between the micronutrient with LAB group and placebo group was not significant. It was concluded that the intervention with heat-inactivated *L. acidophilus* had a negative effect in these children [16]. The second trial investigated the effect of heat-inactivated *L. casei* GG compared with viable *L. casei* GG in children with acute rotavirus diarrhea. The clinical recovery from rotavirus diarrhea was equal in both groups [17].

Cow's Milk Allergy Management

Kirjavainen et al. [18] compared the effect of an extensively hydrolyzed whey formula (EHWF) supplemented with live or killed *Lactobacillus* GG (LGG) and non-supplemented EHWF among 35 infants (mean age, 5.5 months) with atopic eczema and cow's milk allergy. The authors reported significant reductions in scoring atopic dermatitis (SCORAD) scores in the EHWF group, EHWF/viable LGG group, and EHWF/heat-inactivated LGG group (baseline vs. end of a 1-month intervention). Compared with the EHWF group, the decrease in SCORAD scores was significantly higher in the EHWF/viable LGG group ($P = 0.02$). However, this study performed only a post hoc analysis. No adverse events were reported in the EHWF group or EHWF/viable LGG group. However, compared with these two groups, administration of EHWF/heat-inactivated LGG resulted in a significantly higher risk of diarrhea ($P = 0.05$).

Non-clinical Outcomes

A number of studies evaluated additional non-clinical effects [17, 19–22]. For example, the use of fermented formula reduced fecal pH values. However, whether fecal pH reduction is beneficial has not been well established. The same applies to other stool parameters, such as fecal IgA levels and bifidobacteria levels.

Postbiotics in Adults

In adults, data on postbiotics are limited. However, orally administered, inactivated lactic acid bacteria were found to have efficacy for eradication of *Helicobacter pylori* (inactivated culture of *L. acidophilus*) [23] and reduction of IBS symptoms in an observational study (inactivated *Lactobacillus* LB) [24]. More recently, it was shown to have efficacy in an RCT (heat-inactivated *Bifidobacterium bifidum* MIMBb75) [25], for chronic unexplained diarrhea (heat-killed *Lactobacillus acidophilus* LB) [26], and for suppression of the negative impacts of stress (heat-inactivated, washed *Lactobacillus gasseri* CP2305) [27]. These results are not directly applicable to pediatric populations, but they reveal directions for future research.

Conclusions

As the term "postbiotics" is new, the applications of postbiotics to infant nutrition and pediatric gastroenterology are still developing. While there have been some promising results, overall, there is limited evidence to suggest that post-

biotics have health benefits for pediatric conditions. As yet, no clear recommendations for or against the use of postbiotics in pediatrics can be formulated. The effects of postbiotic supplementation have been studied mainly in fermented infant formulas. Overall, the safety and potential harms of postbiotic interventions remain poorly understood. Further multicenter studies are necessary to determine the effects and safety of different postbiotics.

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Part II

Hepatology

Normal Liver Anatomy and Introduction to Liver Histology

56

Maesha Deheragoda

Normal Liver Development

The liver commences development between the 3rd and the 4th weeks of gestation. Development commences as an outpouching, called the liver diverticulum, which arises from the ventral endodermal layer of the distal foregut [1]. The endodermal cells in the cranial part of the diverticulum penetrate the septum transversum to become the hepatocytes and intrahepatic biliary system. The septum transversum is the mesodermal plate separating the thoracic and abdominal cavities and will form the connective tissue of the hepatic stroma and the capsule. Hepatocytes proliferate within the septum transversum and become organized into cords around developing sinusoids, which are derived from vitelline vein branches that perforate the septum transversum. The septum transversum eventually separates from the endoderm-derived hepatocytes and becomes the diaphragm.

The caudal part of the hepatic diverticulum that did not invade the septum transversum becomes the gallbladder, cystic duct and common bile duct by the 5th week of gestation [2]. The intrahepatic bile ducts begin developing between the 5th and 9th weeks of gestation and arise from the limiting plates of the forming hepatoblast cords in the mesenchyme, adjacent to the portal vein branches. Hepatoblasts flatten to become the ductal plate – a continuous layer of biliary-type cuboidal cells. By remodelling to form discrete tubular spaces at around 12 weeks of gestation, the intrahepatic bile duct system will develop. This process starts around the hepatic hilum and progresses towards the periphery of the liver, completing at around 6 weeks postnatally [3, 4]. SOX9 appears to be a key component coordinating the timing of biliary development, with Notch, Wnt, TGF-β, Hippo-Yap and FGF signalling pathways forming downstream signalling pathways [5, 6]. HNF6 and HNF1β appear to be involved in remodelling of the ductal plate [7]. Failure of remodelling results in ductal plate malformation, seen in congenital malformations of the intrahepatic biliary tree [8]. Antigen expression on bile canaliculi, spaces lined by specialized membranes into which hepatocyte bile is exported, is seen at 14–20 weeks of age [9]. Hepatocellular bile acid synthesis begins at 5–9 weeks and bile secretion in the 12th week [10]. However, the exchange of biliary solutes across the placenta is important in foetal life as the canalicular transport and hepatic excretion are still immature.

The liver receives a dual blood supply through the portal vein (which supplies venous blood from the intestines, pancreas and spleen) and an arterial supply from the hepatic artery. During initial stages, the blood flow into the sinusoids of the developing liver comes via the two vitelline veins and the right and left umbilical veins, until the 5th week when the right umbilical vein involutes and the left umbilical vein becomes the main blood supply from the placenta [11]. The venous pattern of the liver is dependent on involution of parts of the venous system and joining of the umbilical veins and vitelline venous system. In the 7th week, the portal vein is already formed from the anastomosing channels of the vitelline veins. Intrahepatic branches of the vitelline vein become the portal venous system. The intrahepatic segments of the portal vein and its branches become surrounded by mesenchyme and will become the portal tracts. Progressive branching of the right and left portal vein branches within the liver creates the terminal portal venules and inlet venules which open into the sinusoids. The sinusoidal blood drains into the centrilobular venules and eventually through the hepatic veins into the inferior vena cava (which is formed from the proximal end of the right vitelline vein). The umbilical vein gives branches to the left liver and drains into the ductus venosus, a structure that connects it to the inferior vena cava [12]. The hepatic artery arises from the celiac axis. At the porta hepatis, it divides into right and left hepatic arteries. These undergo branching to form terminal hepatic arterioles, which communicate with the hepatic sinusoids [11].

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The umbilical circulation closes at birth, with cessation of umbilical venous flow by 20 days. The reduction in venous flow results in collapse of the ductus venosus, within a week of postnatal life. The umbilical vein becomes the ligamentum teres and the ductus venosus becomes the ligamentum venosum. Development of the arterial system mirrors that of the bile ducts and reaches maturity at 15 years of age [13].

Innervation of the liver appears in the later stages of embryonic development, continues to mature postpartum and comprises sympathetic and parasympathetic fibres [14], which enter through the porta hepatis. The capsule is supplied by a few branches of the lower intercostal nerves.

Liver haematopoietic activity starts from the 6th week, and by the 12th week, the liver is the main site of haematopoiesis, until the third trimester of intrauterine life [11]. It diminishes slowly after birth [15]. The liver at birth will have periportal deposits of iron, and these gradually disappear at 3–4 months of age. Residual periportal iron after 4 months of age may reflect poor growth but can also be seen in certain disorders if present in larger quantities – for example, cystic fibrosis, alpha-1 antitrypsin deficiency, urea cycle disorders, citrin deficiency, mitochondriopathies, peroxisomal disorders, congenital disorders of glycosylation and tyrosinaemia, to name a few. Periportal copper-binding protein may also be seen as a physiological finding up to 4 months of age. Intrahepatic haematopoiesis can be seen physiologically within the liver up to 12 months of age. Beyond this point, it may indicate the presence of a haematological disorder, particularly when also associated with immature myeloid forms.

Coordinated growth of the embryonal liver is dependent on a large number of growth factors such as hepatocyte growth factor (HGF), transforming growth factor beta (TGF-β), tumour necrosis factor alpha (TNF-α) and factors involved in the Wnt-signalling pathway [16]. The topic is beyond the scope of this chapter and covered in several excellent reviews [16–19].

Normal Liver Macroanatomy

The liver is the largest visceral organ and continues to grow until around 15 years of age, doubling in size in the first year of life. In adulthood, it will weigh ten times greater than its weight at birth. The liver is divided into right and left lobes and two small central quadrate and caudate lobes. The liver is covered by the visceral peritoneum, except for the 'bare area' on the posterior surface, which is in direct contact with the diaphragm. At the porta hepatis, the hepatic artery and portal vein enter the liver, and the common hepatic duct exits the liver. The liver is connected to the diaphragm and to the anterior wall of the abdomen by five ligaments: the falciform, the coronary and two lateral (triangular) ligaments, the fifth being a fibrous cord (the round ligament) corresponding to the obliterated umbilical vein.

For the purpose of facilitating surgical resections and radiological interpretation, the liver macroanatomy can be subdivided into eight segments based on the intrahepatic distribution of the portal and hepatic veins [20]. The liver is divided into two functional lobes of similar sizes, separated on the liver surface by an imaginary line going through the inferior vena cava sulcus and the middle of the gallbladder fossa. The right lobe is further divided into posterior and anterior sectors and the left lobe into medial and lateral parts. Eight segments, with almost independent blood supply, are thus formed as follows: (I) caudate lobe, (II) superior subsegment of the left lateral segment, (III) inferior subsegment of the left lateral segment, (IV) left medial segment, (V) inferior subsegment of the right anterior segment, (VI) inferior subsegment of the right posterior segment, (VII) superior subsegment of the right posterior segment and (VIII) superior subsegment of the right anterior segment [20]. Segments II, III and IV are drained by the left hepatic vein; the middle hepatic vein drains blood from segments IV, V and VIII; and the right hepatic vein drains segments V–VIII. The inferior hepatic vein drains the caudate lobe (segment I) directly into the inferior vena cava.

Liver Microanatomy and Considerations When Interpreting Histological Findings in Paediatric Liver Biopsies

The terms 'lobule' and 'acinus' are often used by pathologists to describe the distribution of changes, which may in turn give clues about the underlying aetiology. Some scoring systems such as the modified Ishak scoring system also rely on the use of these systems to describe and score patterns of injury [21]. The lobule is a hexagonal structure which places the hepatic venule in the centre and the portal tracts at the corners [22] (Fig. 56.1). The hepatic acinus is a subdivision of the lobule into triangles, with the portal venule at the apex and the portal tracts at the base [23] (Fig. 56.1). The acinus is divided into three zones: zone 1, which is located in the vicinity of the portal tract; the mid-zone (zone 2); and zone 3, situated close to the terminal hepatic venule (Fig. 56.1). The concept of the liver acinus is also useful when considering functional heterogeneity that exists along the portocentral axis. Periportal hepatocytes have a higher capacity for gluconeogenesis and fatty acid metabolism and those in perivenous areas have a higher capacity for detoxification [24]. Gene expression levels can vary across the acinus or can be restricted to particular parts of the acinus. For example, glutamine synthetase, an enzyme involved in ammonia metabolism, is normally restricted to acinar zone 3 and under control of the Wnt/beta-catenin signalling pathway [25].

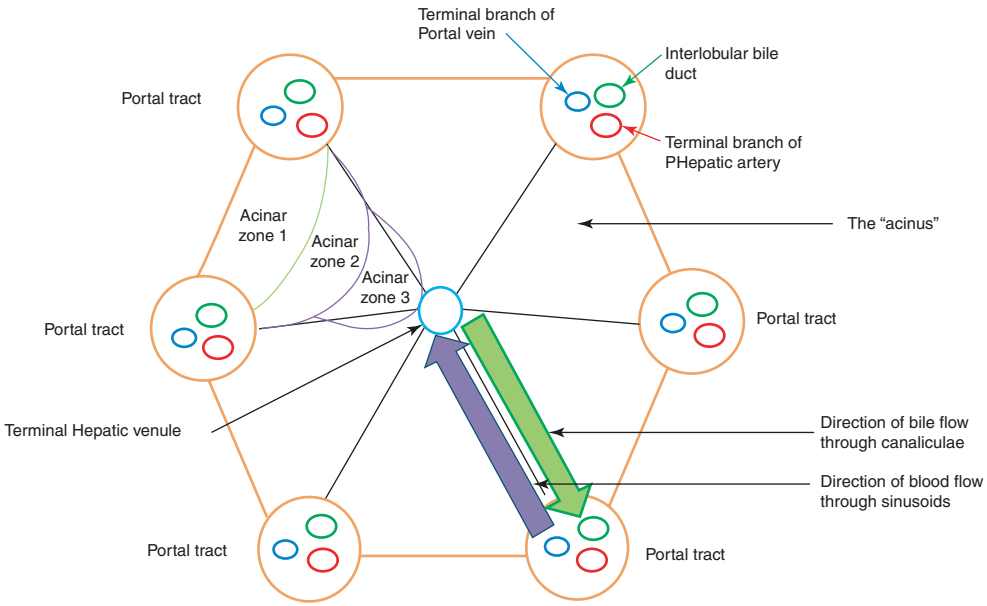

Fig. 56.1 Schematic diagram of the structural and functional components and anatomical relationships of the hepatic lobule (orange hexagonal outline) and the acinus

Non-physiological changes in the immunohistochemical expression pattern of glutamine synthetase are useful when diagnosing disorders of vascular flow or tumours [26]. Enzymes can also change in level according to changes in the hormonal or metabolic state.

Under light microscopy, hepatocytes are polygonal in shape with an approximate diameter of 25 μm. The cell membrane can be divided into three aspects, with different characteristics: a basolateral aspect facing the sinusoidal lumen, a canalicular aspect, which delimits the canalicular space and the lateral aspect between the other two. The nucleus is centrally located. Most hepatocytes are mononuclear, although binuclear hepatocytes are often found. In the neonate and up to 1 year of age, multinucleate hepatocytes can also be seen – often referred to as 'giant cells'. The hepatocyte cytoplasm is abundant and eosinophilic. Periportal hepatocytes in children and adolescents show physiologically vacuolated nuclei. Hepatocytes are arranged in cell cords of variable thickness to around 5 months of age, when they form two-cell-thick cords. At approximately 5 years of age, they adopt the adult pattern of one-cell-thick cords.

Terminal portal tracts show a 1:1 pairing of hepatic arteries and terminal bile ducts. There are approximately two artery/bile duct pairs per single portal vein [27]. There remains a connection of bile ducts with the hepatic parenchyma through bile ductules. Ductules are lined by bile duct epithelial cells (cholangiocytes) derived from the ductal plate. The canals of Hering are an extension of the ductules that penetrate the interface between the portal tract and hepatic cords, with one half of the circumference composed of bile duct epithelial cells and the other half comprising hepatocytes. They may penetrate into the lobule up to a third of the way towards the terminal hepatic venule [28]. The Canals of Hering are thought to contain resident hepatic stem/progenitor cells throughout life [29]. Biliary stem/progenitor cells are thought to be located within peribiliary glands along larger intrahepatic bile ducts [30]. Any significant injury to either bile ducts or hepatocytes at the interface is accompanied by proliferation of the canals of Hering – ductular compartment – leading to a ductular reaction [11].

The bile canaliculi drain bile across the lobule towards acinar zone 1 (see Fig. 56.1) and connect to the bile ducts through the canals of Hering. Downstream propulsion of bile is provided by a combination of a contractile subapical hepatocellular actin network and the fluid pressure generated by active secretion of biliary solutes and fluid [31]. These membranes are highly specialized structures involved in bile transport into the canalicular space and contain proteins involved in bile transport and/or membrane function. Tight junctions also exist between hepatocytes and probably play a role in maintenance of specialized membrane function. Defects in bile acid transporters and tight junction proteins can lead to familial intrahepatic cholestatic liver disorders.

The hepatic sinusoids are specialized vascular channels passing between hepatocyte cords, optimizing contact between the blood contents and the parenchymal elements of the liver. They are lined by fenestrated endothelium. Kupffer cells – specialized liver macrophages – are present on the internal sinusoidal surface [3]. Approximately two thirds of the intrahepatic blood supply is from the portal venules and the remaining third from the hepatic arterial blood supply. Flow of blood into the sinusoids is controlled by sphincters in arteriolar walls and inlets and outlets of sinusoids. The composition of blood within the sinusoids can be arterial, venous or a mixture of the two, depending on sphincteric activity and the distance of the sinusoid from

the portal tract [32]. The space between the endothelial cells and the hepatocytes is defined as the space of Disse, which contains the hepatic stellate cells (Ito cells). They store retinoids, can contract affecting the sinusoidal calibre and can produce extracellular matrix participating to fibrogenesis when activated. Blood from the sinusoids enters the terminal hepatic venule and enters the inferior vena cava via the hepatic veins.

A knowledge of the development of the liver combined with timings of physiological changes described above will aid accurate diagnosis of the paediatric liver biopsy.

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Diagnostic Procedures in Paediatric Hepatology

57

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Imaging of Cholestatic Jaundice

Neonatal

Neonatal jaundice that persists longer than 2 weeks demands clinical, laboratory and radiological investigation, as early diagnosis of surgically treatable conditions leads to favourable outcomes. The role of radiology in the work-up of conjugated hyperbilirubinemia is to make a distinction between obstructive cholestasis due to structural biliary anomalies and non-obstructive conditions such as genetic, metabolic, endocrine or infective disorders, in which imaging investigations are often normal at presentation.

High-resolution ultrasonography is the imaging modality of choice in the screening of these patients and provides the radiologist with a non-invasive, non-ionizing tool, independent of liver function tests.

The ultrasound approach to these patients needs to be systematic, with thorough examination of the liver parenchyma, gallbladder, bile ducts, portal venous system, spleen and pancreas. In particular, the examiner should concentrate on the intra- and extrahepatic bile ducts, in order to detect dilatation. The common bile duct (CBD) should not exceed a calibre of 1 mm in neonates, 2 mm in infants up to 1 year of age, 4 mm in older children and 7 mm in adolescents [1, 2]. The gallbladder needs to be evaluated in its length (normal range between 1.5 and 3 cm) and shape and for the presence of gallstones, inspissated bile and irregular, thickened walls. Evaluation of portal vein patency and direction of flow, presence of collaterals as well as splenic length and ascites is needed to assess signs of portal hypertension. Finally, the size and echogenicity of the pancreas and the calibre of the pancreatic duct (which needs to be <1–2 mm) should also be recorded [3, 4].

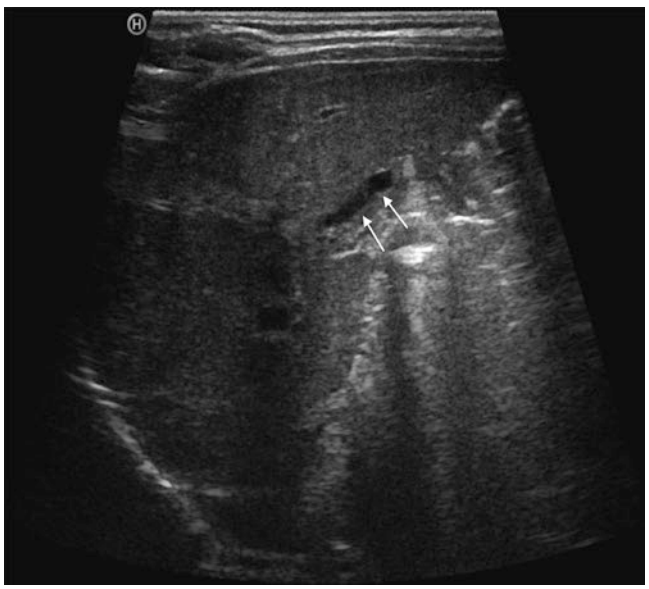

Fig. 57.1 B-mode US of the liver of a patient with biliary atresia shows an abnormal small gallbladder (1.2 cm in length) with irregular walls (*arrows*)

The absence of dilated ducts, a small abnormal gallbladder with irregular walls and hyperechoic fibrous tissue at the porta hepatis (triangular cord sign) are suggestive but not pathognomonic features of extrahepatic biliary atresia (Fig. 57.1). Signs of biliary atresia splenic malformation (BASM) syndrome also need to be sought, such as polysplenia, situs ambiguous, interrupted IVC, pre-duodenal portal vein, anomalous hepatic artery supply and intestinal malrotation (Fig. 57.2); these patients may require MRI supplemented by angiographic sequences for detailed vascular delineation, crucial for pre-operative planning. Infants with biliary atresia can present with end-stage liver disease, and

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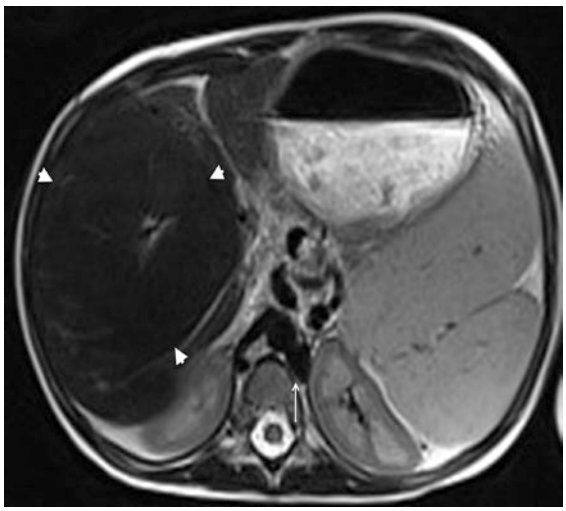


Fig. 57.2 T2W MRI image of a patient with BASM. The liver is cirrhotic with a large central regenerative nodule (central plate hypertrophy, *arrowheads*) and atrophied left lobe and posterior segments of the right lobe. There is polysplenia and interrupted IVC with hemi-azygous continuation (*arrow*)

therefore initial US can detect coarse nodular liver parenchyma, retrograde flow in the portal vein, ascites, varices and splenomegaly. In biliary atresia, hepatic scintigraphy will show normal uptake of radiotracer in the liver but failure of biliary excretion after 24 h (Fig. 57.3); however, neonatal hepatitis could also show absent bowel activity due to poor hepatocellular function. Therefore phenobarbital is administered prior to the procedure to enhance hepatocellular function. Confirmation of diagnosis is normally achieved with liver biopsy, which will differentiate between biliary atresia and neonatal hepatitis, which is a diagnosis of exclusion.

When dilated ducts are detected, the differential will include choledochal malformation, inspissated bile syndrome and Caroli with congenital hepatic fibrosis. In these instances, the patient needs further evaluation to define the anatomy; historically, this was achieved by means of direct percutaneous cholangiography (PTC) or endoscopic retrograde cholangiopancreatography (ERCP), but the implementation of MR imaging with heavily T2w cholangiography

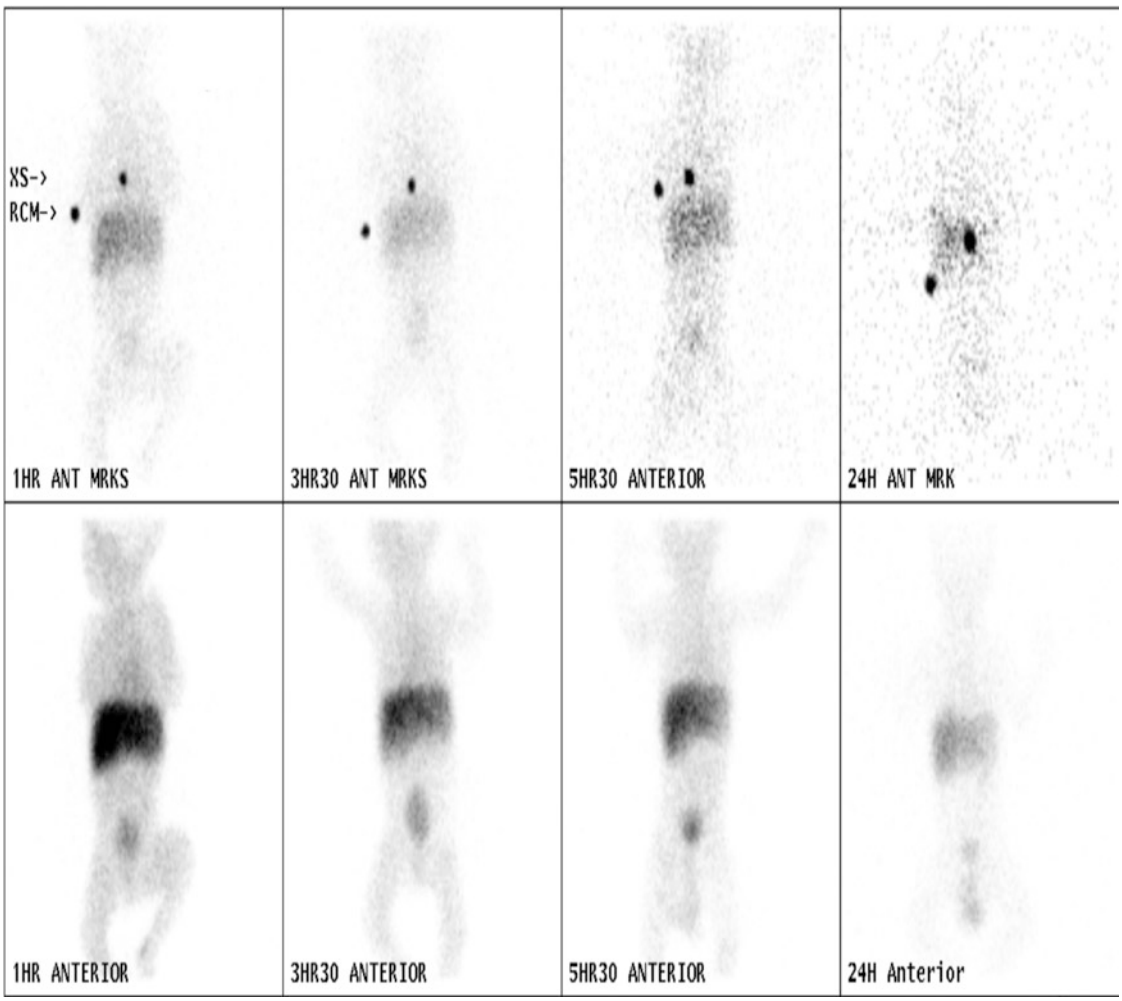


Fig. 57.3 Nuclear medicine hepatobiliary function study demonstrates accumulation of radiotracer in the liver with no evidence of uptake in the intra- or extrahepatic biliary tree. There is no radiolabelled bile seen within the bowel at the 24-h delayed planar image. Normal physiologic uptake is seen within the urinary bladder

sequences has allowed the radiologist to achieve the diagnosis confidently and in a non-invasive way [5].

Magnetic resonance cholangiopancreatography (MRCP) can in fact classify the different types of choledochal malformations and reveal anomalies of the pancreatico-biliary junction (more often associated with type I cysts) with possible complications, such as calculi within a long common channel (Fig. 57.4).

In case of Caroli, MRCP will show multifocal cystic dilatation of the intrahepatic bile ducts, possibly containing filling defects, representing calculi; MR can demonstrate non-invasively that these cysts communicate with the biliary tree, thus excluding autosomal dominant cystic liver disease and biliary hamartomas (Fig. 57.5). A specific MR finding of Caroli is the "central dot sign", a portal vein branch protruding into the lumen of a dilated duct, which enhances with gadolinium [6].

ERCP is not routinely indicated but may still be required in doubtful cases or as a therapeutic option in those patents where MRCP has identified an obstructed biliary system due to inspissated bile or choledocholithiasis or in a very select case of suspected biliary atresia before laparotomy.

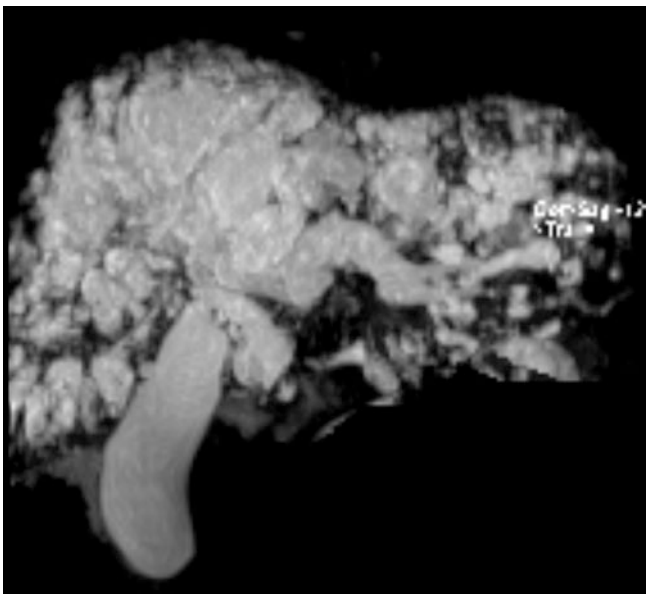


Fig. 57.5 Coronal maximum intensity projection (MIP) MRCP shows numerous cystically dilated intrahepatic bile ducts that communicate with the biliary tree. The common and main hepatic bile ducts are markedly dilated

Older Children

Jaundice in older children can be caused by hepatocellular disease (acute or chronic) and obstructive causes, and imaging investigations can distinguish these entities and often establish the aetiology of many chronic conditions and acquired or developmental biliary disorders.

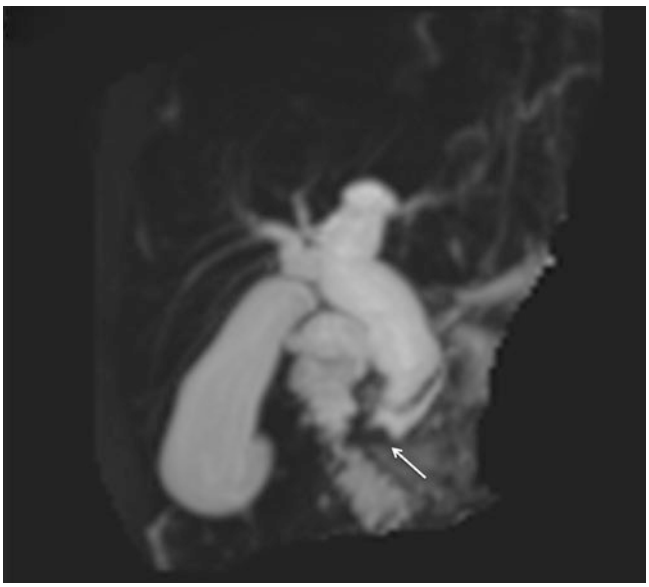


Fig. 57.4 MRCP MIP reconstruction of the biliary tree showing a type of choledochal malformation with an impacted calculus within a long common channel (*arrow*)

Ultrasound findings in acute hepatitis are normally non-specific, with US demonstrating hypoechoic parenchyma, increased periportal reflectivity due to oedema and thickened gallbladder walls.

In the context of chronic liver disease, radiology can confirm the clinical diagnosis demonstrating coarse, heterogeneous liver parenchyma and abnormal liver architecture as well as signs of portal hypertension (Figs. 57.6 and 57.7).

Medical causes of jaundice or liver disease in older children include Wilson's disease, cystic fibrosis, glycogen storage disorder, tyrosinaemia and alpha-1 antitrypsin deficiency. In all these conditions, US will show non-specific changes with hyperechoic liver parenchyma; however, these patients are prone to develop focal liver lesions (such as adenomas and, importantly, hepatocellular carcinomas), and in this instance, US is extremely useful as a non-invasive, radiation-free surveillance test. In addition, some of these conditions have specific imaging features, such as multiple small nodules with low signal intensity in T2 on MR in Wilson's disease and focal biliary cirrhosis with periportal fibrosis seen on US and MR in cystic fibrosis.

Calculi, benign strictures (as seen in primary sclerosing cholangitis, PSC) and neoplasms (which will be discussed separately) are all obstructive causes of jaundice in older children.

Calculi are seen in US as hyperechoic foci with posterior acoustic shadowing, mobile when detected within the gallbladder; these are seen as signal voids on MR. Inspissated bile is again hyperechoic in US but does not cause posterior shadowing.

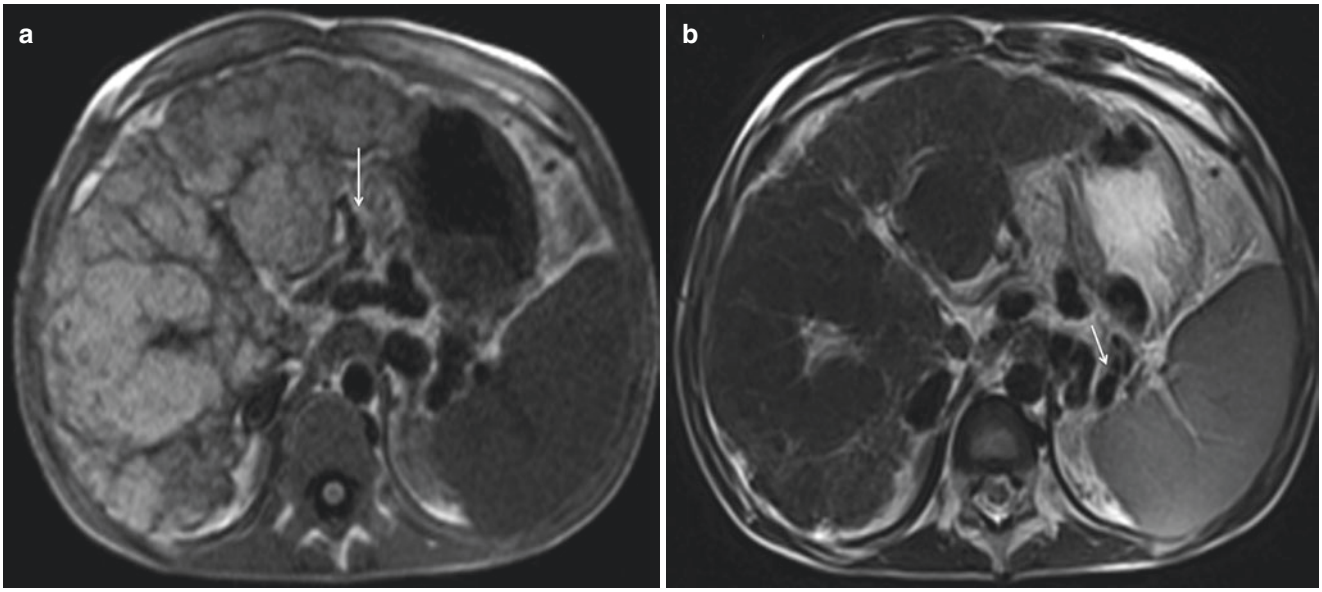


Fig. 57.6 MRI T1W (**a**) and T2W (**b**) images of a cirrhotic liver with markedly abnormal contour and varying size-regenerative nodules throughout. The portal vein is occluded, and there are numerous short gastric and splenic varices (*arrows*)

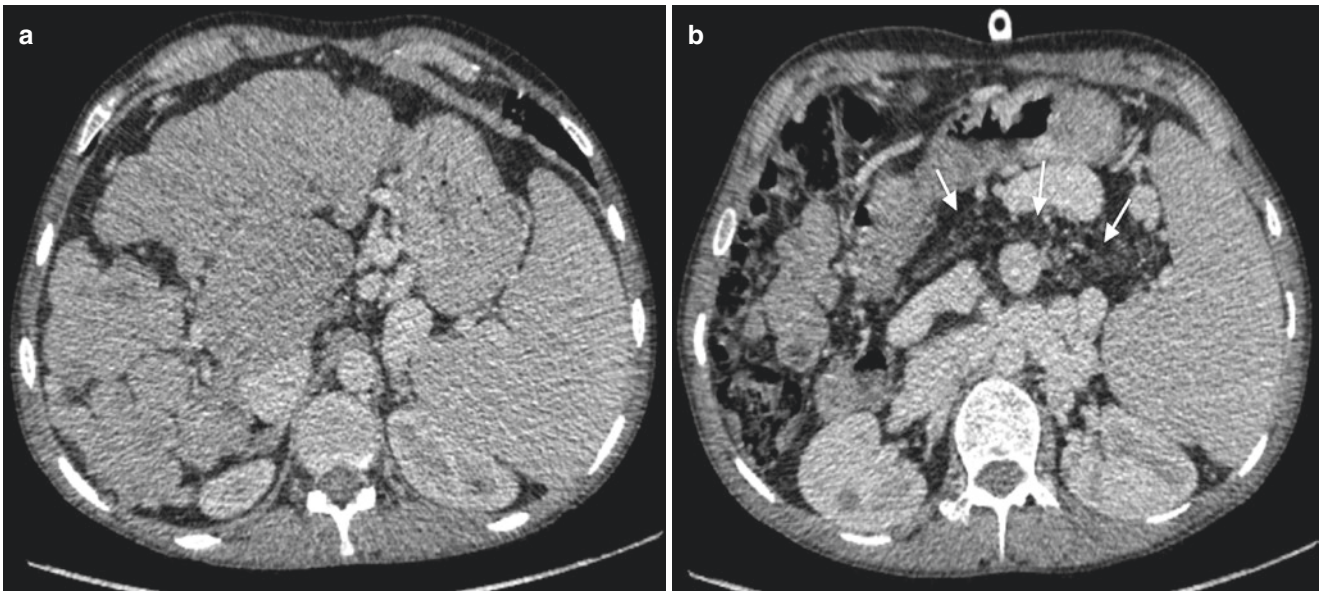


Fig. 57.7 CT of a patient with cystic fibrosis and chronic liver disease. (**a**) Nodular cirrhotic liver and signs of portal hypertension, splenomegaly and varices. (**b**) Fatty infiltration of the pancreas (arrows)

Primary sclerosing cholangitis (PSC) is characterized by inflammation and fibrosis of the biliary tree, which results in cholestasis with progression to secondary biliary cirrhosis and hepatic failure. Histologically, the intrahepatic bile ducts are surrounded by cuffs of inflammatory cells and fibrosis, which results in segmental dilatation of the peripheral ducts alternated with narrow or obliterated segments, reflected in the imaging findings. The radiological diagnosis of PSC is often challenging and needs confirmatory clinical, biochemical and histologic findings. Traditionally, ERCP and PTC have been used as standard imaging procedures for the radiological diagnosis of PSC; however, MRCP has nowadays replaced these techniques.

Ultrasound remains the first-line investigation in these patients and shows segmental duct dilatation, irregular thickened walls of the CBD (Fig. 57.8), lymph nodes at the porta hepatis and heterogeneous reflectivity of the liver parenchyma (depending on the stage of disease). In established chronic liver disease, there is coarse echotexture with nodularity and relative atrophy of the right lobe with hypertrophy of the caudate and lateral segment of the left lobe.

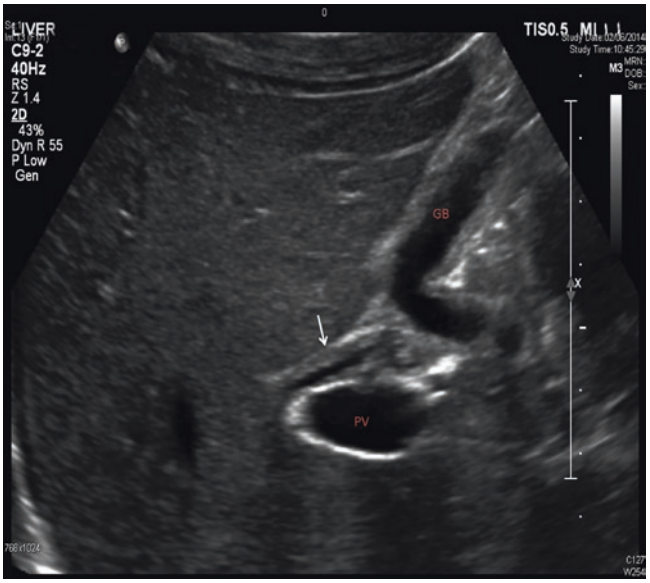


Fig. 57.8 US of the liver of a patient with PSC shows thickened wall of the common bile duct (*arrow*). *PV* portal vein, *GB* gallbladder

MRCP can depict ductal irregularity, stricturing, focal dilatation, beading and pruning affecting the intra- or extrahepatic biliary tree (Fig. 57.9). Post-contrast dynamic MR sequences should also be included to look for enhancement of thickened walls of the extrahepatic ducts and cholangiocarcinoma.

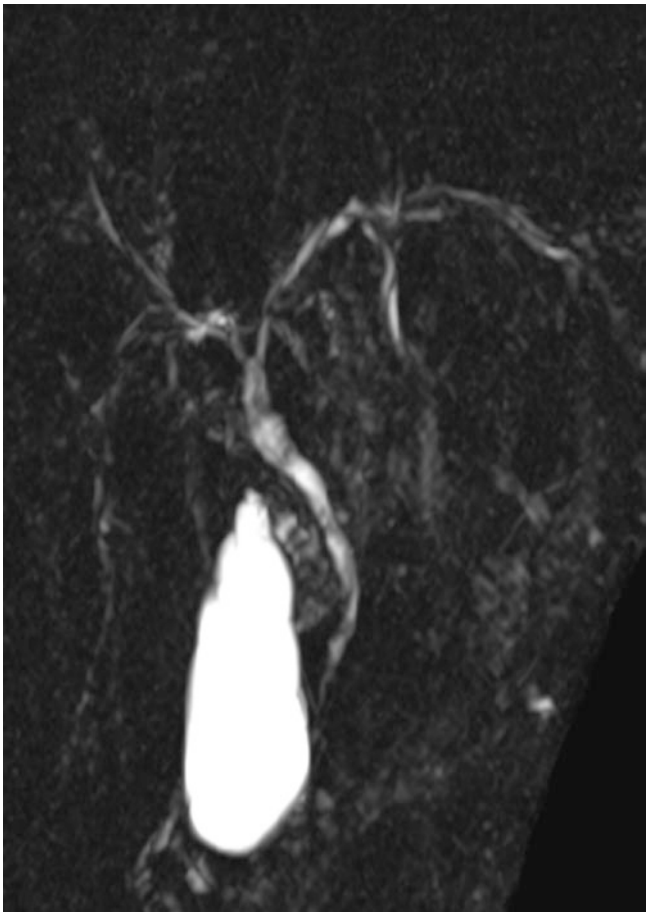


Fig. 57.9 MRCP MIP reconstruction of the biliary tree showing irregularity of the walls of the intra- and extrahepatic bile ducts, with focal structuring, in keeping with PSC. *MIP* maximum intensity projection

Congenital and Acquired Vascular Disorders

Portal Hypertension

The aetiology of portal hypertension can be classified as cirrhotic and non-cirrhotic. The latter can be prehepatic (portal vein thrombosis), intrahepatic (portal vein sclerosis, congenital hepatic fibrosis, steatosis, nodular regenerative hyperplasia, veno-occlusive disease) or suprahepatic (Budd-Chiari). Depending on the aetiology, different radiological findings will be present. Liver function tests may be normal, and the diagnosis of portal hypertension relies on demonstration of splenomegaly and formation of portosystemic collaterals.

In cirrhosis, prehepatic and pre-sinusoidal portal hypertension, the role of radiology is to determine the extent of involvement of the portal venous system, as this would influence the therapeutic options, which include surgical portosystemic shunt, Rex bypass, transjugular intrahepatic portosystemic shunt (TIPS) and ultimately liver transplantation.

The radiological approach to children with portal hypertension starts again from US with Doppler, which can detect low velocity, retrograde or absent flow in the main portal vein and intrahepatic divisions. A patent umbilical vein, splenomegaly, with varices and ascites can also be easily seen on US, however, cross-sectional imaging is used as a "roadmap" for the evaluation of the portal system and collaterals pathways. CT and MRV can also demonstrate cavernous transformation of the portal vein, seen as a typical "beaded" appearance at the porta hepatis, which consists of venous channels within and around a previously stenosed or occluded portal vein [7]. This can occur within a few weeks after a thrombotic event, even in the case of partial recanalization of the thrombosed segment, and can extend to the intrahepatic branches. Cystic and pericholecystic dilated veins can also be seen. CT performed in the arterial phase often shows patchy areas of high density at the periphery of the liver parenchyma, and this is due to a peripheral increase of the arterial inflow to supply the areas not reached by the cavernous portal vein.

Intra- and Extrahepatic Vascular Shunts

Intrahepatic communications between portal vein, hepatic artery and hepatic veins are rare, and the most common,

although often difficult to demonstrate, are small arterioportal shunts seen in cirrhotic livers. Large connections include portosystemic shunts (intra- and extrahepatic), arterioportal shunts and arteriovenous shunts/malformations.

The origin of portosystemic shunts is still controversial and congenital, genetic (such as trisomy 21) and acquired causes (such as in cirrhosis and post-traumatic) have been postulated [8]. These shunts have been historically classified into intrahepatic and extrahepatic; however, some authors discourage this classification and suggest to use an anatomical classification, which is aimed to establish the origin of the shunt (main portal vein, its tributaries or intrahepatic branches) and its systemic termination, the type of communication (end to side or side-to-side) and the number of connections. A distinction should be made between these shunts and a persistence of the ductus venosus [9].

The aim of radiology is to detect the shunts, delineate the anatomy, and assist planning and monitoring of interventional/surgical corrections and follow-up. Doppler US is the modality of choice to achieve this: direct communication between a portal branch and hepatic vein or the ductus venosus can be readily demonstrated, often due to enlargement and tortuosity of the vessels involved; the flow is usually continuous but triphasic flow can be seen in the portal branch. In case of the main portal vein-IVC connections, there is low velocity flow or non-visible intrahepatic portal vein, and the liver is usually decreased in size. Cross-sectional imaging is performed routinely to confirm the diagnosis and define the anatomy prior to intervention or surgery; in addition, shunts between the splenic vein or superior mesenteric vein and the IVC may be easily missed on US. CT and MR are also useful to characterize focal liver lesions, often found in associations with these shunts.

Angiography is often part of the work-up of these children, either as a therapeutic option or prior to surgery to detect non-visible portal vein/portal vein branches. A balloon occlusion catheter is placed in the shunt and pressures are measured before and after occlusion to evaluate how closure will be tolerated. In addition, angiography can investigate if there is continuity between left and right intrahepatic portal venous radicles, crucial to establish feasibility of Rex shunt.

Arterioportal shunts may be congenital (in Rendu-Osler disease) or acquired (post-traumatic, post-liver biopsy, cirrhosis). On CT, small arterioportal shunts in cirrhosis appear as small, wedge-shaped, peripheral areas of increased attenuation with early portal venous filling in the arterial phase and uniform attenuation in the portal venous phase. In the presence of large arterioportal shunts or fistulas, there is early and marked enhancement of the main portal vein or segmental branches during the arterial phase. At Doppler US, large shunts will manifest with pulsatility of the portal vein flow [10].

Connections between the hepatic artery and systemic veins are rare and can be seen in congenital arteriovenous malformations (AVM), HCC and large haemangioendotheliomas; on Doppler US, altered waveforms of the hepatic vein can be seen in severe AVM, whereas CT will show asymmetrical, early filling of the hepatic vein.

Budd-Chiari Syndrome

Budd-Chiari syndrome is the clinical manifestation of hepatic venous outflow obstruction at any level of the hepatic veins, IVC or right atrium; this could be primary, caused by an endoluminal thrombus or membrane or secondary, when the occlusion is due to non-vascular material or from extrinsic compression. On US, there is narrowing and lack of visualization or thrombosis of the hepatic veins/IVC, with absent or monophasic flow at colour Doppler. There is enlargement of the caudate lobe, ascites and signs of portal hypertension with retrograde flow in the portal vein and splenomegaly.

On cross-sectional imaging in the acute setting, occlusion of the hepatic veins with severe ascites is the typical finding. There is patchy, decreased peripheral enhancement of the liver due to portal and sinusoidal stasis and higher enhancement of the central parenchyma and caudate lobe, which compresses the IVC (Fig. 57.10). In subacute Budd-Chiari syndrome, portal vein thrombosis can develop as the result of reduced portal flow caused by blockage of the outflow. Finally, in chronic stages, multiple regenerative nodules can be seen, and on MR these are bright on T1-weighted images and strongly hypervascular on post-gadolinium sequences. The nodules are predominantly isointense or hypointense relative to the liver on T2-weighted images [11].

Imaging of Transplant Liver

Liver transplantation is the treatment of choice for children with end-stage acute or chronic liver failure in which other therapies have failed or are not available. The successful development of novel surgical techniques of segmental, split, auxiliary and living donor transplantation, together with advances in organ preservation, immunosuppressive therapy and adequate choice of donor organ, has led to reduced rate of complications. However, these new surgical techniques have also brought with them new potential complications: in living-related transplants, for example, when the vascular pedicle may be too short, an autologous iliac artery conduit can be used, leading to a potential higher risk of vascular complications.

Early recognition is crucial to the successful management of these complications, and a multimodality imaging

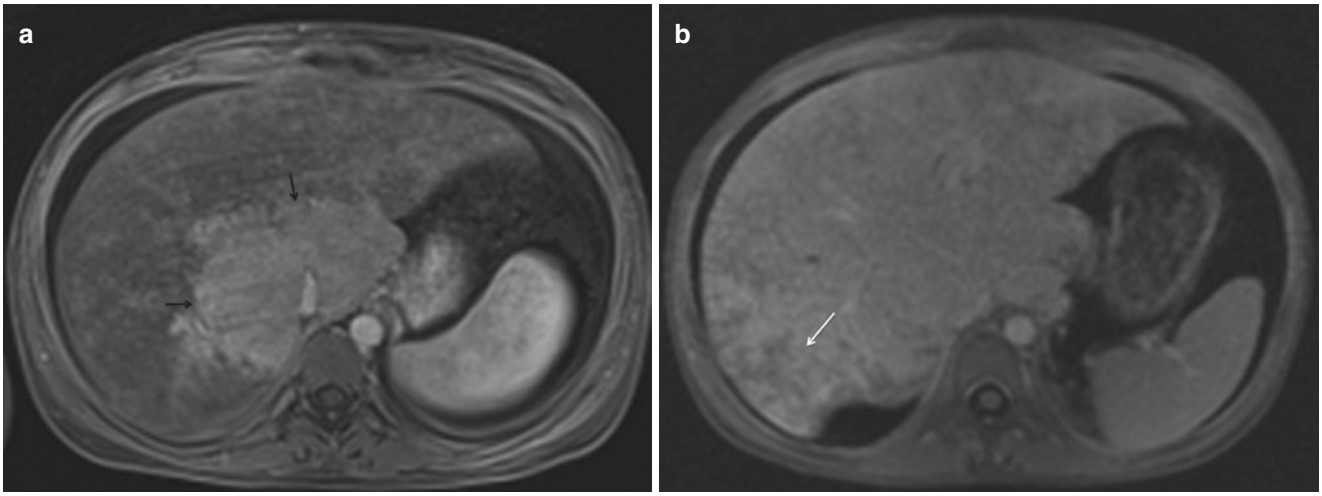


Fig. 57.10 MRI post-gadolinium T1W images of a patient with Budd-Chiari. In the acute phase (**a**), there is significant enlargement of the caudate lobe (*black arrows*), which enhances normally versus the poorly vascularized peripheral parenchyma. The IVC is compressed by the caudate lobe. In the subacute phase (**b**), there are signs of paraseptal necrosis (*white arrow*) with preserved central enhancement

approach is most effective to achieve the diagnosis, except in case of graft rejection, in which radiology has no role.

Serial ultrasound is the screening modality of choice immediately postoperatively and can be easily performed at the patient's bedside; however, ultrasound has inherent limitations, and, even though the use of ultrasound contrast agents can improve its sensitivity, it depends on the expertise of the operator, and cross-sectional imaging is often necessary in inconclusive cases. Conventional angiography is now limited to those cases in which endovascular treatment is required.

We will illustrate the imaging appearances of the main, most common complications following liver transplant.

Hepatic Artery

A normal hepatic artery waveform shows a rapid systolic peak followed by a continuous diastolic flow; the resistive index (RI) should range between 0.5 and 0.8, except in the immediate postoperative period (<72 h), in which the RI can be higher. An increased RI is associated with older donor age and prolonged ischemia time.

Hepatic artery thrombosis is a feared complication and a major cause of graft loss due to fulminant hepatic necrosis; in the long term, this complication can also lead to ischaemic cholangiopathy, as the hepatic artery is the only arterial supply to the bile ducts in liver grafts. Children are particularly at risk, due to hepatic artery size discrepancy between recipient and donor.

On colour and Doppler US, complete loss of arterial signal at the porta hepatis and within the liver suggests hepatic artery thrombosis. A tardus parvus waveform (prolonged systolic acceleration time and low flow velocity with RI < 0.5) at the porta hepatis or in the intrahepatic arteries indicates significant impairment of hepatic arterial perfusion, usually as a result of either arterial stenosis (uncommon) or thrombosis with collateral arteries (Fig. 57.11): collateral vessels from the Roux loop may form rapidly following hepatic artery thrombosis; therefore surveillance with Doppler US in the immediate postoperative days is vital. Conversely, false-positive results can be found in case of hepatic oedema or systemic hypotension [12]. In case of inconclusive findings on US but still high clinical suspicion of hepatic arterial insufficiency, a CT angiogram with thin-slices acquisition should be performed, which will show abrupt interruption of the hepatic artery and ischaemic/necrotic parenchyma in case of thrombosis and focal narrowing with decreased hepatic perfusion in case of stenosis. Ischaemic lesions can liquefy and complicate with infection and abscess formation.

The role of conventional angiography is mostly therapeutic rather than diagnostic, with thrombolysis, angioplasty or stenting (depending on the findings) to allow early revascularization.

Portal Vein

Portal vein complications are relatively rare and normally result from technical problems, such as difference in calibre between donor and recipient, narrow native portal vein (e.g. in biliary atresia) and stretching at the anastomotic site or other factors including previous thrombosis, hypercoagulable states and increased resistance due to inferior vena cava (IVC) strictures.

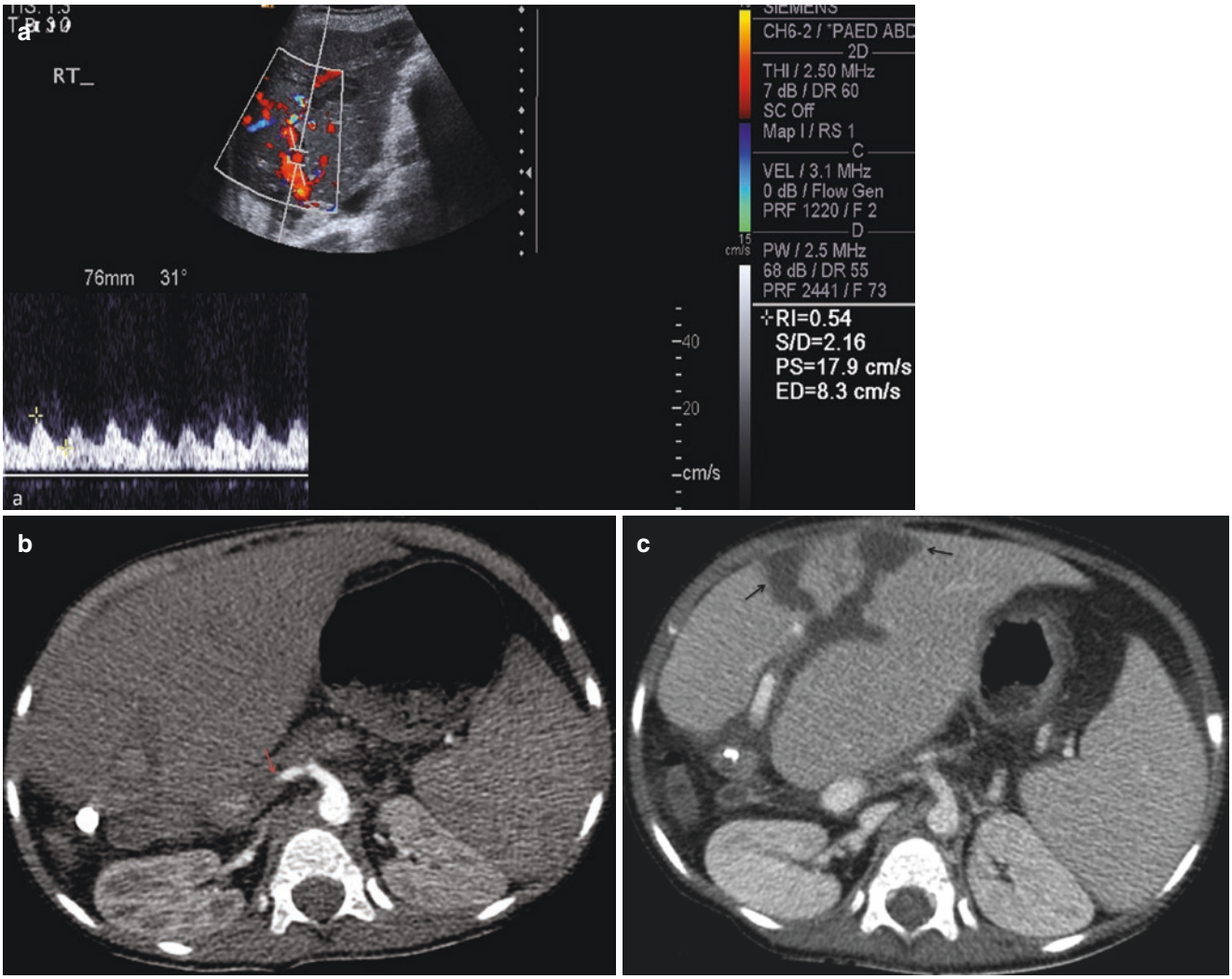


Fig. 57.11 Doppler US scan of a left lateral segment transplant liver. There is a pulsus tardus parvus in a collateral of the hepatic artery with a prolonged acceleration time and reduced peak systolic velocity (17.9 cm/s). (**b**) CT angiogram confirms the finding of a thrombosed hepatic artery (*red arrow*). (**c**) A follow-up CT scan performed 1 month later shows low-attenuation lesions within the parenchyma, in keeping with bile lakes (*black arrows*)

On US, a normal portal vein has smooth walls and an anechoic lumen and on colour and Doppler images shows antegrade/hepatopetal monophasic flow, except in the early postoperative period, where it can show high velocity and turbulent flow.

Portal vein thrombosis manifests with echogenic intraluminal thrombus and lack of flow on colour and Doppler trace, and absent portal vein enhancement is seen on CEUS (Fig. 57.12), in which case either thrombolysis or, more frequently, surgery with thrombectomy or venous graft is performed.

Portal vein stenosis has a later onset and can present with signs of portal hypertension. B-mode US shows focal severe narrowing of the portal vein at the anastomotic site with post-stenotic dilatation; on colour and Doppler images, there is focal aliasing at the stenotic site with an increase of the velocity of three- to fourfold compared to the pre-stenotic segment [12, 13]. These findings can be confirmed with CT or, usually for the late-onset complications, with MR angiography (Fig. 57.13). Symptomatic patients are normally treated with balloon angioplasty or stenting (Fig. 57.14).

IVC

Venous outflow obstruction is a rare complication secondary to either stenosis, thrombosis of the suprahepatic IVC or caval kinking from organ rotation.

The normal Doppler trace of the hepatic vein and IVC is triphasic due to pressure variations during the cardiac cycle;

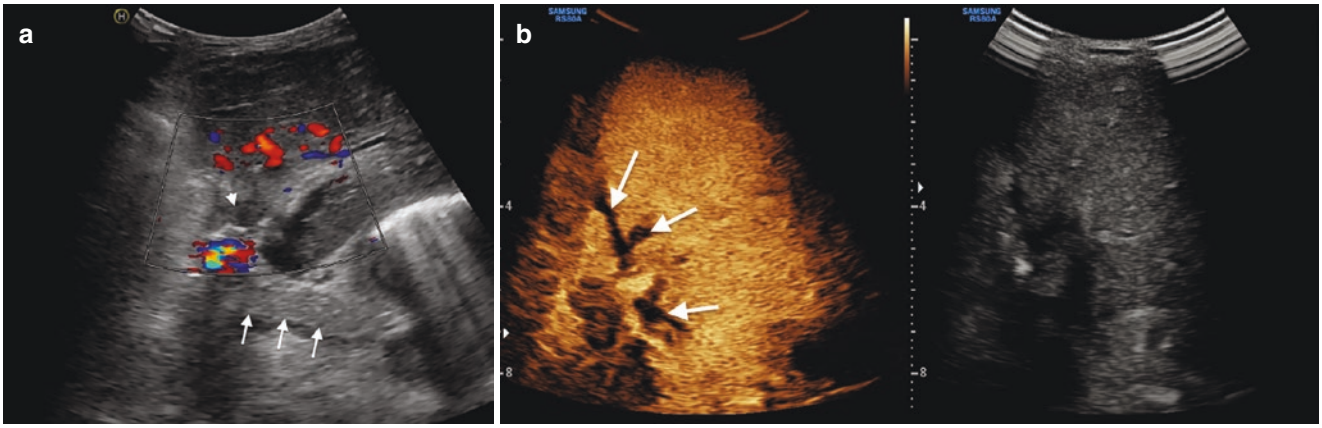


Fig. 57.12 (**a**) US shows an echogenic thrombus within the extrahepatic portal vein (*arrows*), with extension into the intrahepatic portal veins and absent flow on colour Doppler (*arrowhead*). (**b**) CEUS shows the absence of enhancement of the portal vein and branches (*arrows*)

a monophasic waveform is a sensitive but non-specific sign of stenosis, and a triphasic or biphasic waveform can exclude a substantial stenosis. Like the portal vein, stenosis can be seen on US as a focal stricture with impaired flow and prestenotic dilatation of the hepatic vein, whereas echogenic thrombus and complete absence of flow are direct signs of thrombosis. CT or MR is normally needed to confirm US findings or make the diagnosis where US is inconclusive. Cross-sectional imaging can also show signs of Budd-Chiari syndrome with the typical mosaic pattern of perfusion.

Biliary Disorders

Biliary complications are relatively frequent and are a common cause of graft dysfunction; these can often present with ischaemic liver injury as well as cholestasis and cholangitis and include anastomotic strictures, fistulas, stone formation with obstruction, diffuse cholangiopathy and bile leaks.

A Roux-en-Y choledochojejunostomy is the most commonly performed anastomosis to achieve biliary drainage, although an end-to-end choledochal anastomosis may be performed in older children recipient of a whole graft.

US serves as a surveillance test to detect bile duct dilatation, although this is a variable feature, as mild dilatation can be present in the absence of significant obstruction and therefore clinical and laboratory correlation is needed.

MRCP is the modality of choice to investigate cholangiopathy and biliary strictures, the most common biliary complication (Fig. 57.15). This can be due to either fibrotic proliferation and narrowing of the lumen or ischaemic cholangiopathy; multiplanar MR provides anatomical details and helps in planning percutaneous, endoscopic or surgical treatments.

Bile leaks normally occur early and can form perihepatic collections, which are drained percutaneously; treatment options include placement of a stent or biliary reconstruction. Intrahepatic bile leaks are normally secondary to ductal ischaemia.

Liver Masses

Radiological Approach to the Child with a Focal Liver Lesion

The diagnostic approach to children with chronic liver disease or a suspected liver mass should be tailored to the clinical scenario, including the age of the child and serum alpha-fetoprotein (AFP) levels. Not all malignancies produce AFP; however, if serum AFP is elevated, a benign lesion is unlikely. US is the modality of choice in the screening of these children, because of its lack of ionizing radiation and no need for sedation. Some tumours, such as haemangioendothelioma, have typical features on US and Doppler scan and may not require further characterization; however, the vast majority of liver masses need cross-sectional investigation [14, 15]. Contrast Enhanced Ultrasound (CEUS) is a useful and evolving technique for assessing focal liver lesions and has the potential to not only differentiate benign from malignant liver lesions but also establish a diagnosis in benign lesions such as focal nodular hyperplasia, without the need for further cross-sectional imaging [16, 17].

When performing a CT in children, efforts should be made to minimize radiation exposure, and unnecessary phases should be avoided (such as non-contrast scans); when the aim of the scan is surgical planning, a single post-contrast phase should be performed.

If multi-phase post-contrast imaging is required, then MRI should be performed instead of CT. The limiting factor here is the need for sedation in young children; therefore each scan should be performed starting from the most valuable sequences to achieve a definitive diagnosis.

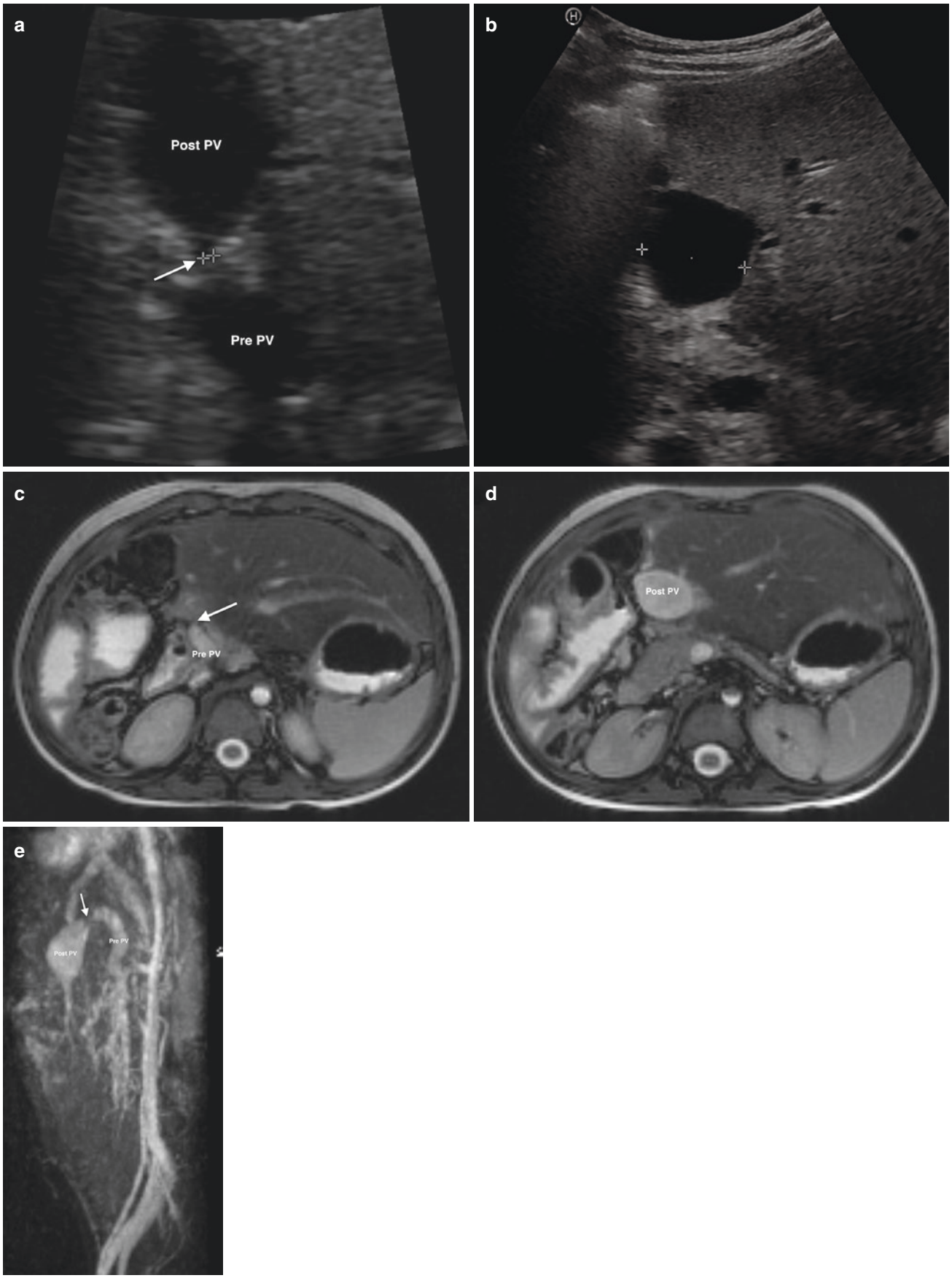


Fig. 57.13 B-mode US of a post-liver transplant patient. (**a**) Anastomotic stricture of the portal vein (*arrow*, 1 mm) (**b**) with post-stenotic dilatation. (**c**) T2W MRI demonstrates the anastomotic stricture (*arrow*) and (**d**) post-anastomosis stricture dilatation. (**e**) MRV MIP sagittal image shows the PV stricture (*arrow*) and post-anastomosis stricture dilatation. Pre-anastomosis portal vein *Pre PV*, post-anastomosis portal vein *Post PV*

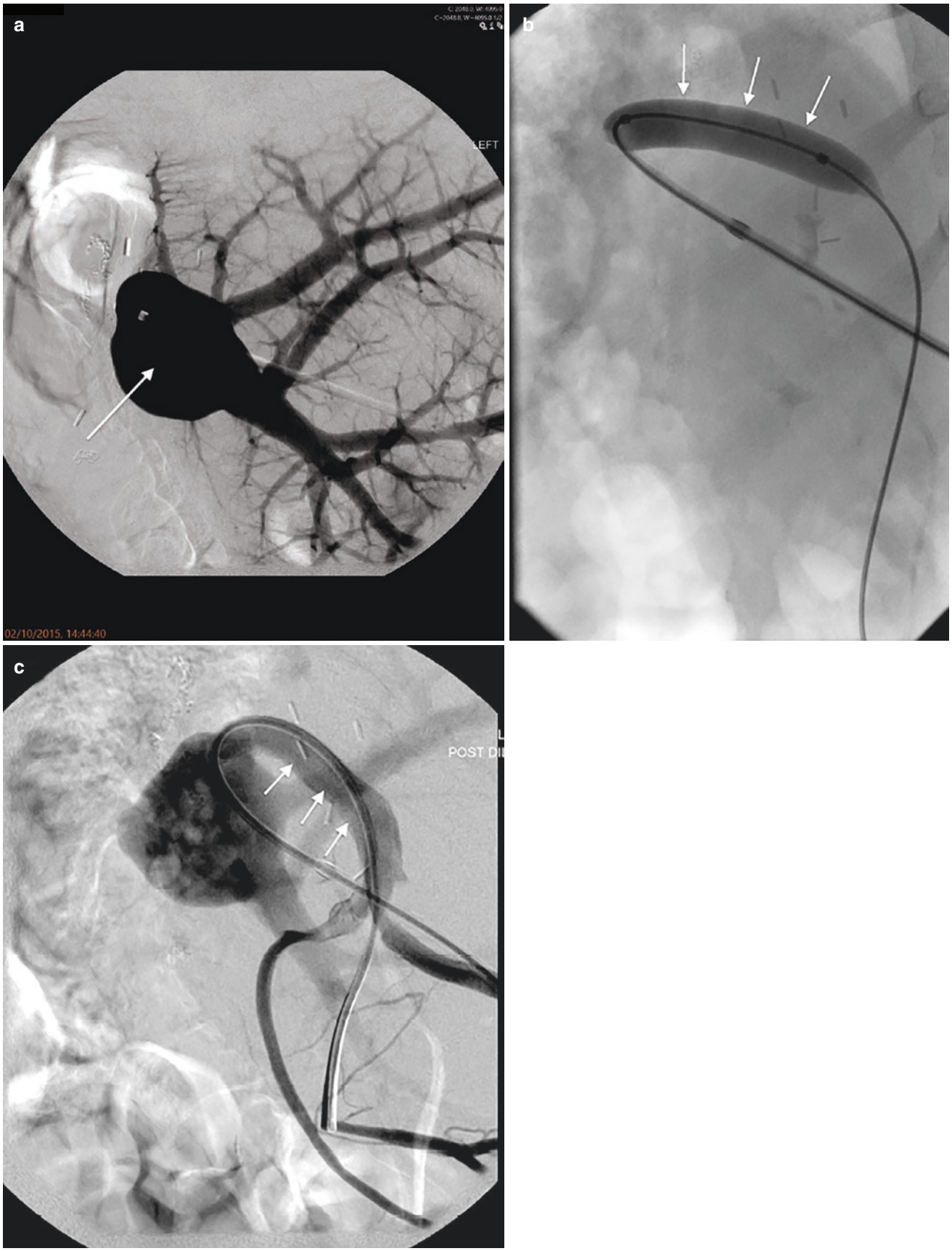


Fig. 57.14 Percutaneous transhepatic portal venogram. (**a**) Post-anastomosis stricture dilatation of the portal vein (*arrow*). (**b**) Balloon angioplasty of the PV stricture (*arrows*). (**c**) Post-balloon dilatation of the PV anastomosis stricture (*arrows*)

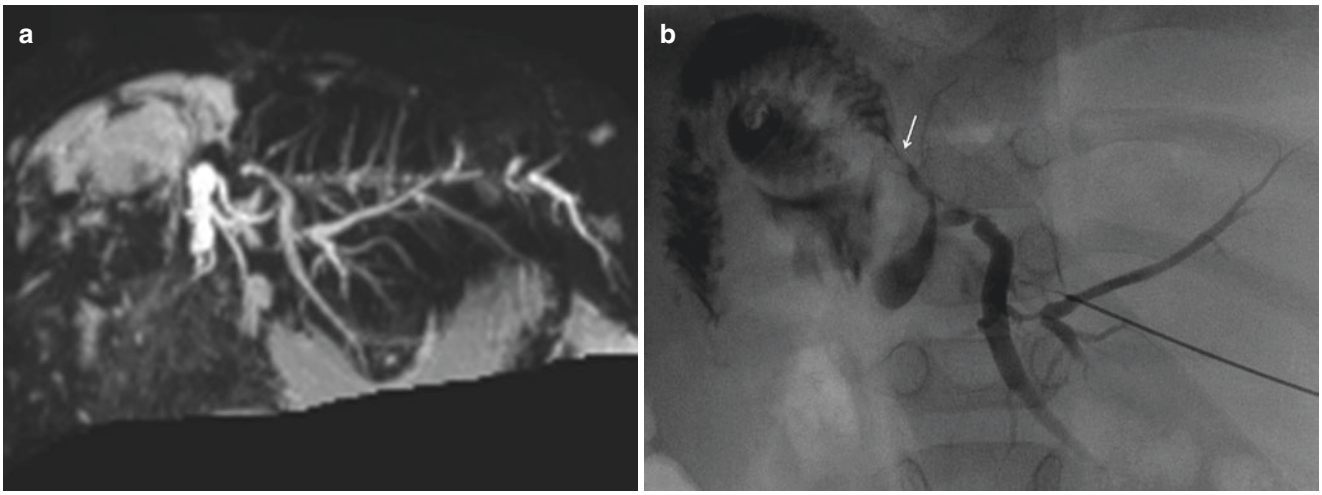


Fig. 57.15 (**a**) MRCP MIP reconstruction of the biliary tree in a left lateral segment transplant liver shows signs of cholangiopathy with an anastomotic stricture of the biliary enterostomy. (**b**) PTC image confirming the MRCP findings of a stricture (*white arrow*). The patient underwent balloon dilatation of the stricture. *MIP* maximum intensity projection

If, at the end of the diagnostic pathway, there is still uncertainty on the nature of a lesion, then imaging-guided (either US or CT) biopsy should be performed.

Benign Tumours

Infantile Hepatic Hemangiomas

Infantile hepatic hemangioma (IHH) is a vascular neoplasm and represents the most common benign tumour in children; the lesions can be either single or multifocal, and its margins may be sharp or ill-defined. In the first 6 months of life, these tumours show progressive growth, followed by spontaneous involution. On antenatal US scan, a hypoechoic liver mass can be detected, sometime together with foetal hydrops and cardiomegaly, which correlate with worse prognosis.

Multifocal lesions are small and uniform in appearance, usually hypoechoic on US within a markedly enlarged liver. Large lesions can have heterogeneous appearance in all modalities, due to central haemorrhage, necrosis, fibrosis and calcification, and prominent vascular channels will be seen on colour Doppler. On CEUS, peripheral nodular enhancement is seen on the arterial phase with centripetal homogenous or incomplete enhancement on venous and late phases (Fig. 57.16).

On cross-sectional imaging, there are signs of high flow, with enlargement of the hepatic artery and veins (due to the presence of arteriovenous shunting) and possibly tapering of the aorta below the origin of the coeliac axis. Like CEUS, similar enhancement characteristics are seen as CT and MRI: peripheral nodular enhancement with "filling in" on the delayed phase. On MR, the lesions have characteristic hyperintense signal on T2W images (Fig. 57.17).

Angiography is now reserved for those cases in which arteriovenous shunts require embolization of the feeding arteries.

Mesenchymal Hamartoma

Mesenchymal hamartomas typically present as solitary tumours of the liver in infants and small children; most commonly, they involve the right lobe of the liver, and the vast majority of these contain cysts, even though they can be either predominantly cystic or solid. The gross pathologic appearance will influence imaging findings: cystic portions are avascular with thin or thick septa, and solid components are hypovascular compared to the normal liver parenchyma. The cysts will be anechoic on US and have high signal on T2W MRI and either low signal (clear cyst contents) or high signal (mucoid contents) on T1W images (Fig. 57.18). CEUS will show enhancement of the wall and septa, which persists on delayed phases.

Focal Nodular Hyperplasia (FNH)

FNH is a benign epithelial liver tumour, uncommonly seen in young children. The tumour is composed of well-differentiated hepatocytes forming nodules divided by

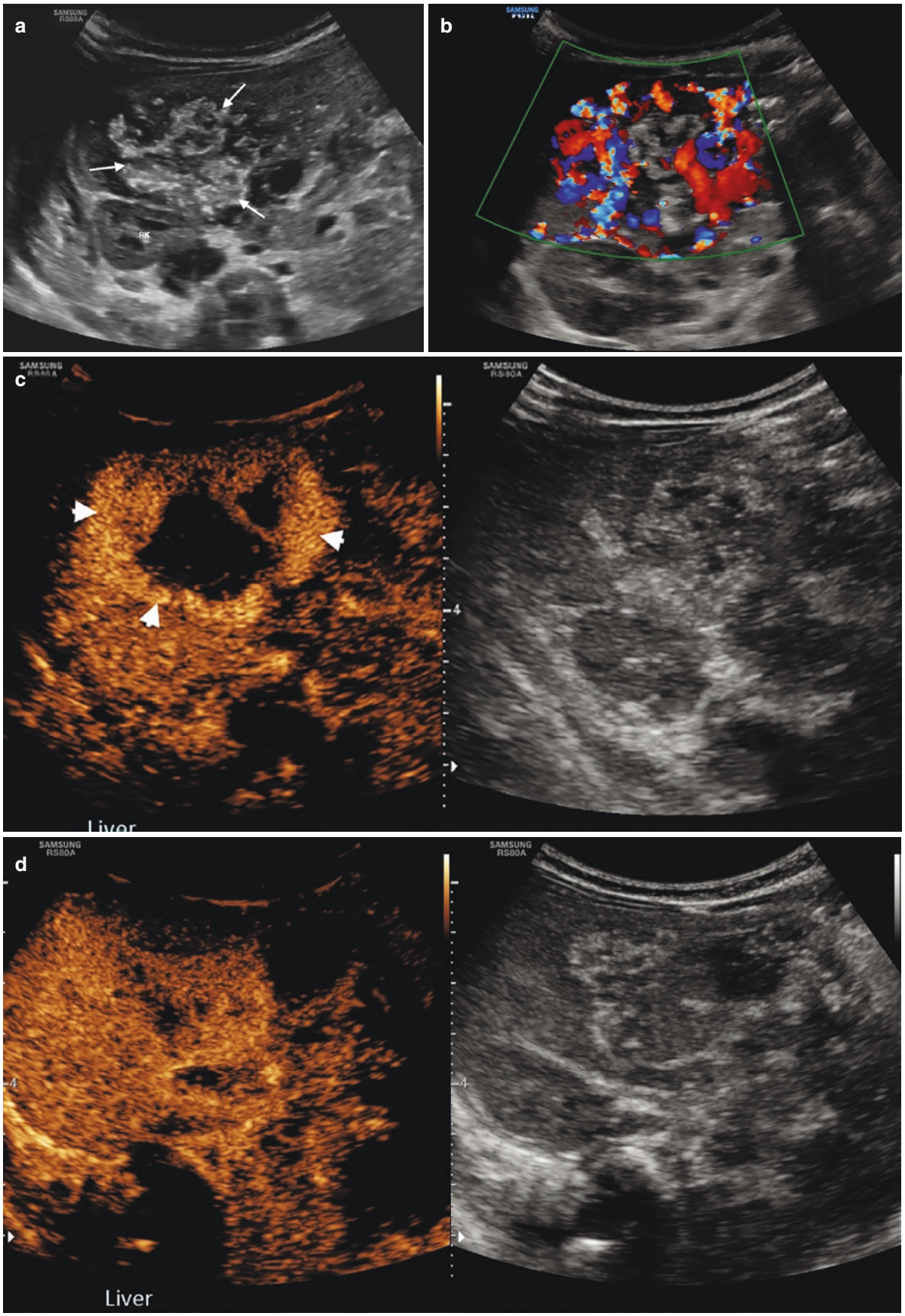


Fig. 57.16 Conventional ultrasound of an infantile hepatic hemangioma (IHH). (**a**) Mixed echogenic mass with solid and cystic components and calcifications with on B-mode ultrasound (arrows). (**b**) Prominent vascular channels are seen on colour Doppler US. CEUS in a different patient demonstrates (**c**) peripheral enhancement on the arterial phase (*arrowheads*) and (**d**) incomplete centripetal enhancement on the venous phase. *RK* right kidney

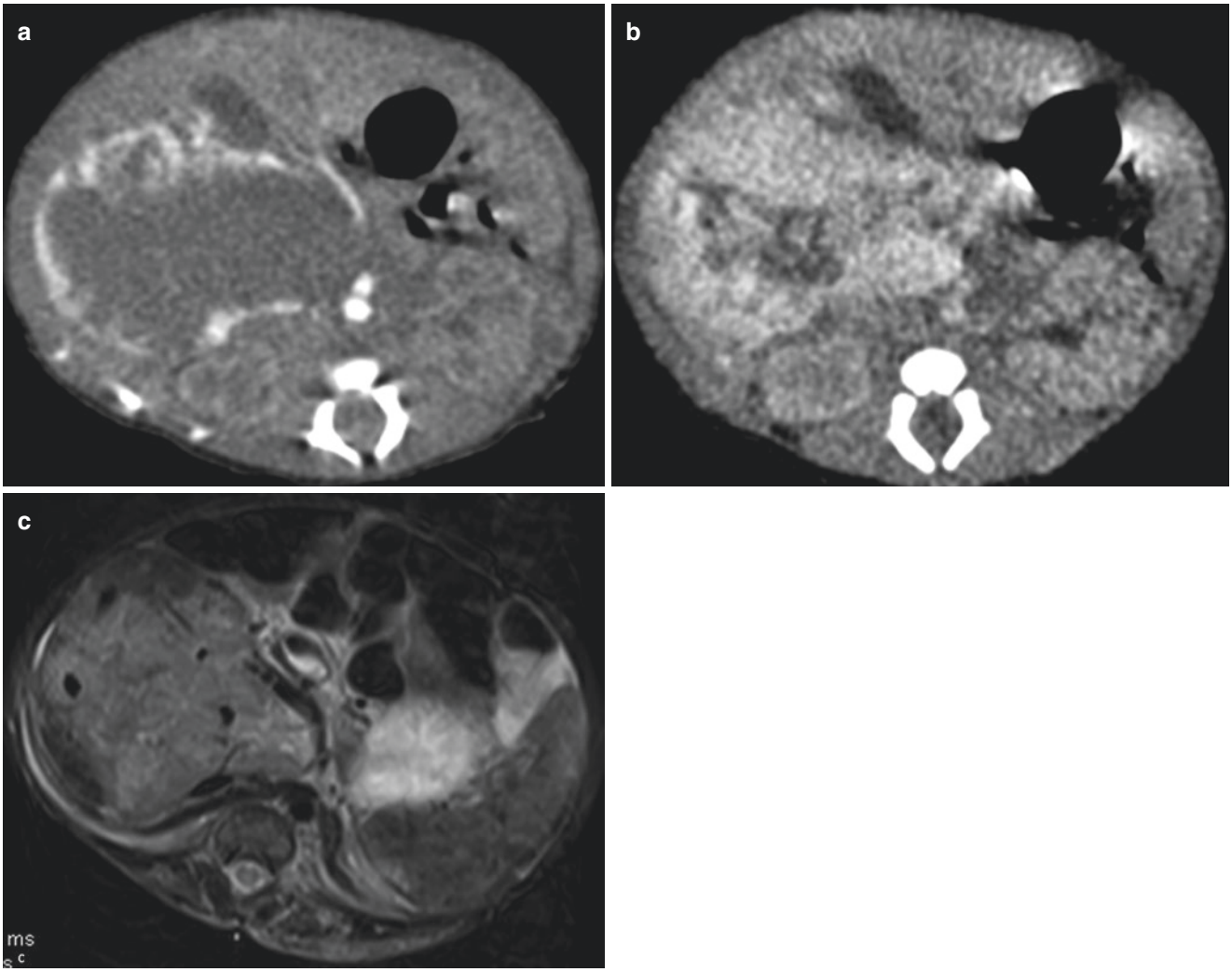


Fig. 57.17 (**a**) CT scan performed in the arterial phase in a new-born baby shows a large IHH in the right lobe of the liver with peripheral enhancement. (**b**) In the portal venous phase, there is centripetal enhancement of the mass. (**c**) A T2W MR image shows the lesion as hyperintense compared to normal liver parenchyma

fibrous septa, which form a characteristic vascular central scar with a stellate shape. It is usually a solitary, well-circumscribed, encapsulated mass but can be multifocal. On US it appears as a homogeneous lesion of variable echogenicity, usually isoechoic compared to normal liver. Central vascularity can be detected at colour Doppler, and CEUS will show a typical pattern of centrifugal, spoke-wheel arterial enhancement followed by uniform enhancement with the rest of the parenchyma in the portal venous and late phases (Fig. 57.19) [17]. This can be confirmed at post-contrast cross-sectional imaging; on MR, similar enhancement characteristics are seen, and the central scar is normally bright in T2W images (Fig. 57.20). In equivocal cases, hepato-specific agents (such as Teslascan or Primovist) show tumour uptake that persists for hours or even several days.

Hepatocellular Adenoma

Hepatocellular adenoma (or hepatic adenoma) is a rare benign neoplasm composed of hepatocytes with increased intracellular fat and glycogen, often prone to intralesional haemorrhage and necrosis. The majority of hepatic adenomas present as solitary well-circumscribed lesions, although in children with glycogen storage disease they can be multifocal.

Hepatic adenomas without haemorrhage will be homogeneous with the rest of the liver in all imaging modalities. Following administration of contrast in CT or MR, the lesions typically display increased enhancement in the arterial phase and uniform enhancement with the rest of the liver in the portal venous and delayed phases; if there is haemorrhage, the lesion will enhance heterogeneously. Similar

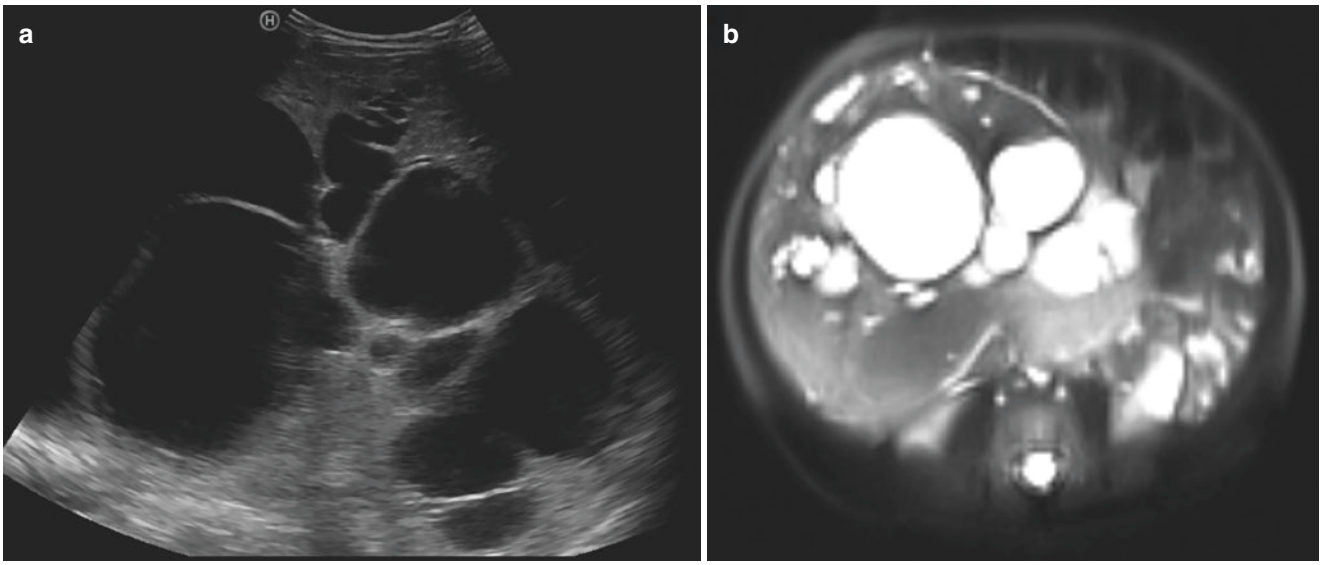


Fig. 57.18 (**a**) US shows a multicystic mass with anechoic cysts and thin intervening septa. (**b**) A T2W MR image shows the lesion with multiple hyperintense cysts

enhancement characteristics are seen on CEUS with mixed or centripetal arterial enhancement, isoenhancing with the liver parenchyma on the venous phase, and without washout on the early and late phase. Lesions with high-fat content will show on MR high signal intensity on T1W and T2W images, with signal dropout on opposed-phase or fat-suppressed sequences.

Nodular Regenerative Hyperplasia (NRH)

NRH is characterized by regenerative nodules surrounded by atrophic liver without a fibrotic component. Various types of small-vessel blood flow alterations are seen in association with NRH, including lymphoproliferative disorders, autoimmune disorders and Budd-Chiari syndrome, and with the use of various medications (steroids, chemotherapeutic agents). The nodules, usually multiple with the tendency to coalesce, are composed of hepatocytes like the surrounding liver; therefore they can be difficult to distinguish from the adjacent parenchyma in all modalities, even after contrast administration.

Malignant Tumours

Hepatoblastoma

Hepatoblastoma is the most common primary hepatic tumour in children, occurring predominantly in children below the age of 5 years. These tumours normally present as large masses often containing haemorrhagic and necrotic areas and speckled or amorphous calcifications; in 90% of the cases, AFP serum levels are elevated. At imaging, hepatoblastomas present as large, well-circumscribed masses with septa; epithelial tumours have a homogeneous appearance, whereas mixed epithelial and mesenchymal tumours are more heterogeneous with variable osteoid, cartilaginous and fibrous components. The primary role of radiology is to assess the tumour for resectability (sections involved, involvement of the caudate, vessel invasion) and staging (the lung being the most common site for metastases): hepatoblastoma should be evaluated using the PRETEXT classification as this will establish treatment regimen [18]. On post-contrast cross-sectional imaging and CEUS, hepatoblastomas can show early avid, inhomogeneous enhancement and early washout (Fig. 57.21); vascular invasion is better demonstrated on contrast-enhanced scans.

Hepatocellular Carcinoma

The main differential diagnosis of hepatoblastoma is hepatocellular carcinoma; HCC however is rare under the age of 5 years, and 50% of cases have a pre-existing liver disease, such as biliary atresia, familial intrahepatic cholestasis, glycogen storage disease type I, tyrosinaemia, Wilson's disease and alpha-1 antitrypsin deficiency.

Unlike in adults, small HCCs are unusual in children, and lesions can have variable appearance on US, with a hypoechoic halo if the tumour has a capsule. Large tumours may contain areas of haemorrhage and necrosis.

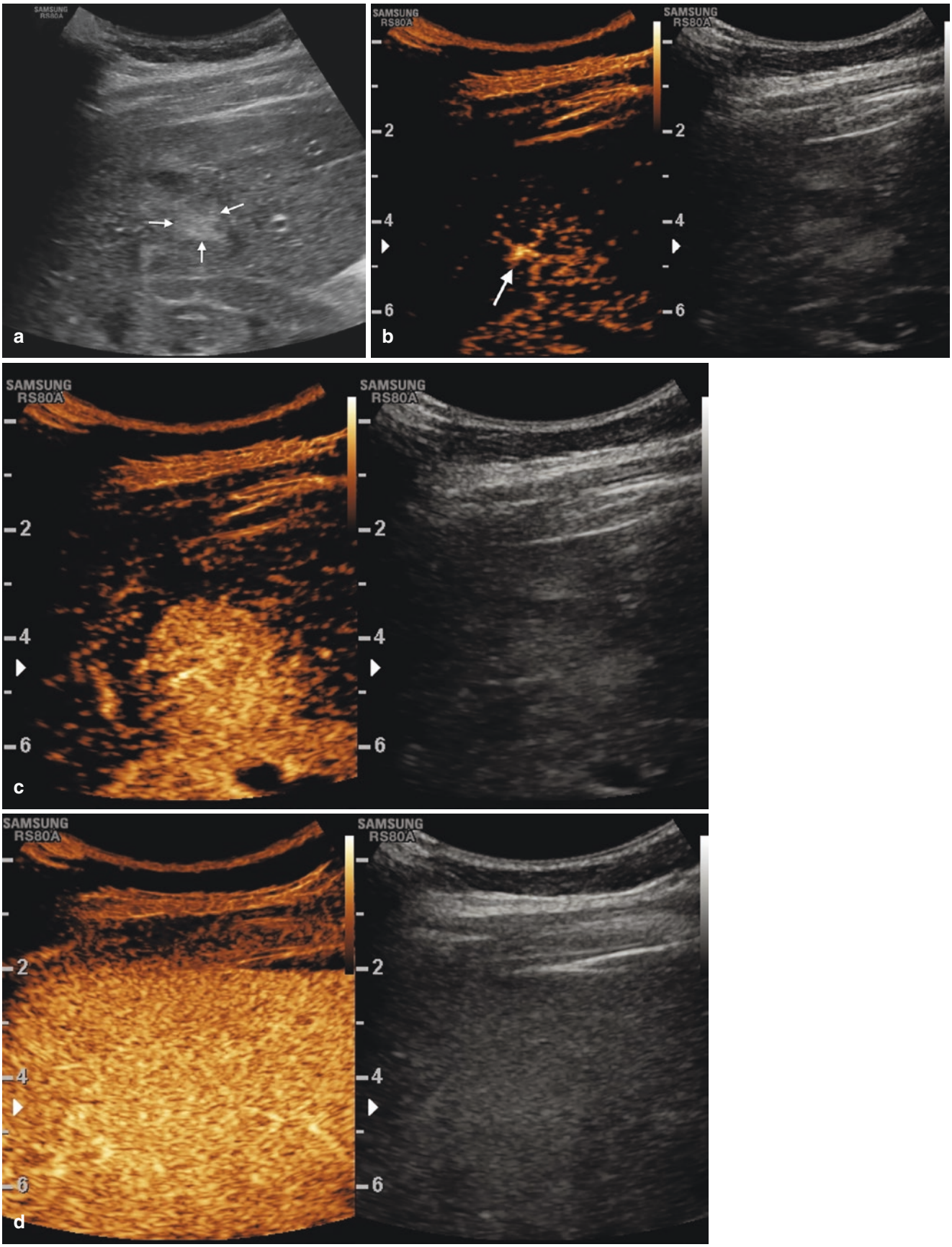
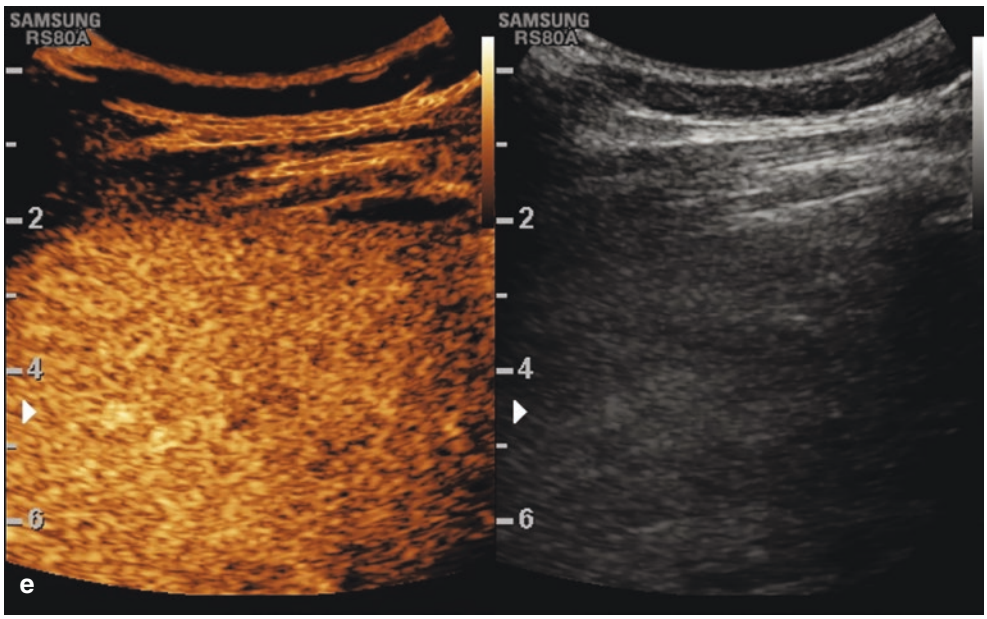


Fig. 57.19 Conventional US of an FNH. (**a**) B-mode shows a well-defined slightly hyperechoic mass (*arrows*). CEUS (**b**) early arterial phase (16 s) demonstrates a spoke-wheel pattern (*arrow*). (**c**) Late arterial phase (25 s) shows centrifugal homogeneous enhancement (**d**) venous (50 s), and (**e**) late phase (67 s) shows isoenhancement with the surrounding liver parenchyma with no evidence of washout

Fig. 57.19 (continued)



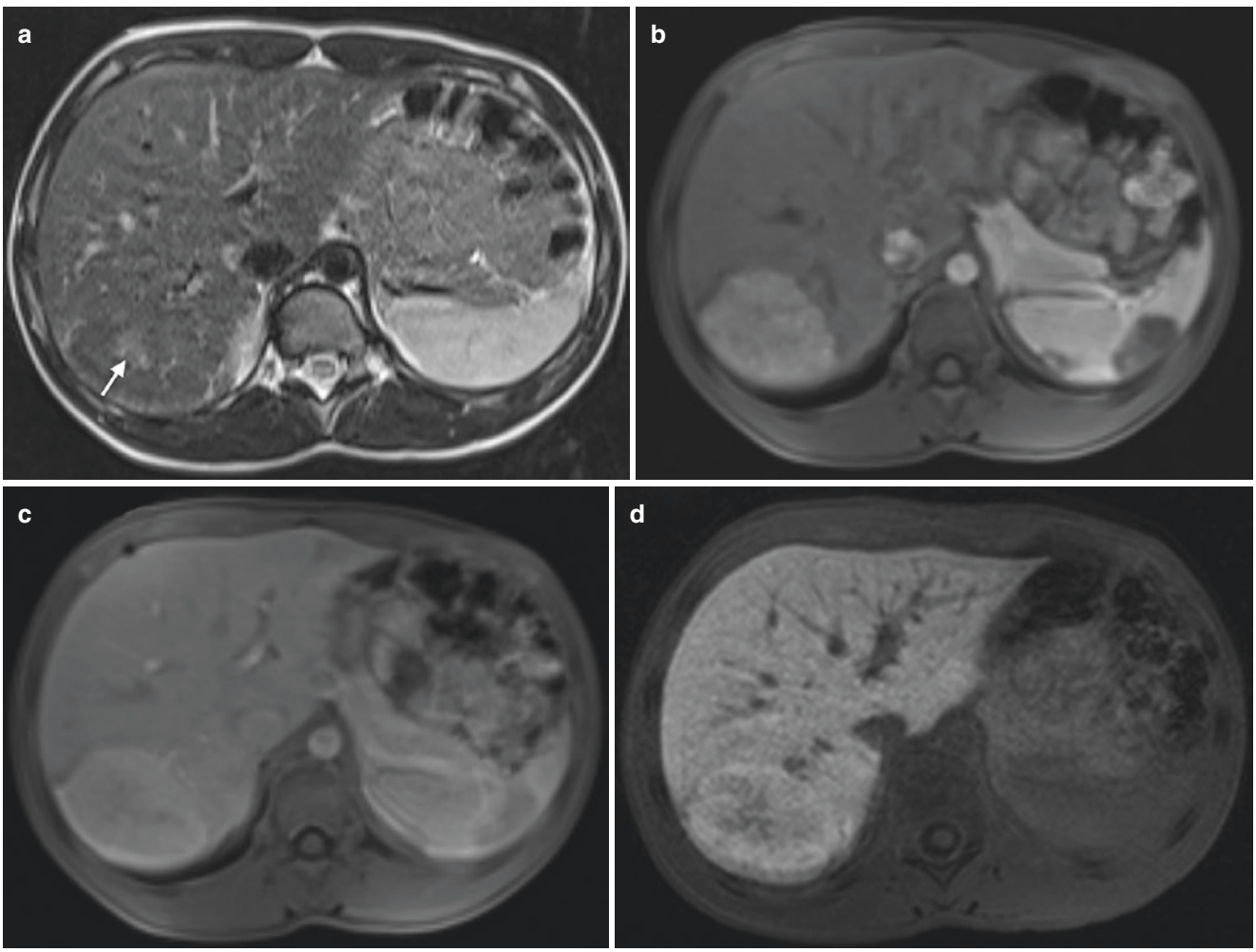


Fig. 57.20 MRI of an FNH. (**a**) A T2W image shows a mildly hyperintense central scar (arrow). Post-contrast T1W FS (**b**) arterial phase shows homogeneous intense enhancement. (**c**) Iso- to mildly hyperintense on the venous phase. (**d**) No evidence of washout on the hepatobiliary phase

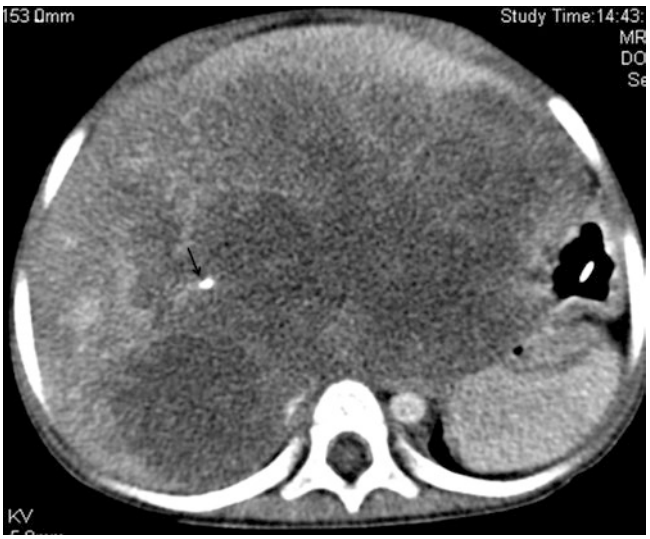


Fig. 57.21 A CT scan performed on the portal venous phase shows a large, poorly enhancing hepatoblastoma involving the whole of the liver; note intralesional calcification (*arrow*)

On post-contrast cross-sectional imaging and CEUS, HCC typically shows early arterial enhancement with washout in the portal venous and delayed phases; portal and hepatic venous invasion may be identified.

Fibrolamellar Carcinoma

Fibrolamellar carcinoma is a slow-growing tumour that occurs in adolescents and young adults. The tumours are normally well demarcated and contain numerous fibrous septa which may separate the tumour into lobules and form a central scar-like structure; haemorrhage and necrosis are rare but calcifications can be observed. US shows a solitary heterogeneous mass; post-contrast CT and MR show avid enhancement in the arterial phase with often lack of enhancement of the central scar. On MR, the scar is typically hypointense in T1 W and T2W images, in contrast with the scar seen in FNH, which is normally hyperintense in T2W images. CEUS may have value in demonstrating similar enhancement characteristic like CT and MR imaging and a central non-enhancing scar in all phases, but there is only limited published evidence to support this [19].

Undifferentiated Embryonal Sarcoma (UES)

UES is a rare tumour that usually presents between 6 and 10 years of age; AFP levels are usually normal and the presentation is non-specific. The tumours show very aggressive local growth with invasion of the diaphragm and lung. These tumours typically show a predominantly solid appearance on US but cystic appearance on CT and MR, due to its prominent myxoid stroma, which contains water. These tumours are usually well-demarcated by a fibrous pseudocapsule, which generally show marked enhancement on post-contrast images.

Angiosarcoma

Angiosarcoma is a rare vascular tumour that can occur in young girls and in children with a previous diagnosis of IHH.

US can show either multiple nodules or a large mass of variable echogenicity. On post-contrast imaging, the lesion often show decreased, heterogeneous enhancement compared to the normal liver with occasionally central or ring enhancement. Haemorrhage or necrosis can be observed.

Embryonal Rhabdomyosarcoma

Rhabdomyosarcoma can rarely arise from the biliary tree, and this form is almost exclusively seen in young children; it spreads by intraluminal extension and causes significant cholestasis. At imaging, a mass can be seen usually at the porta hepatis, with intrahepatic biliary duct dilatation and normal-sized or, if affected by the tumour, enlarged extrahepatic duct (Fig. 57.22). Portal vein displacement without invasion is common. Like other malignant liver lesions, post-contrast enhancement with early washout can be seen on CEUS.

Imaging of Liver Trauma in Children

CT remains the gold standard in the evaluation of blunt abdominal traumas in children; however, due to the risks related to radiation exposure, CT must be reserved for those children in whom there is a high index of suspicion and should be performed following IV injection of contrast, possibly with split-bolus technique. When indicated, CT can provide rapid information regarding intra-abdominal injuries and can guide patient's management towards operative or conservative. Even though US would be preferable to CT given its non-invasive nature and the lack of radiation, published data regarding the use of FAST (Focused Abdominal Sonography for Trauma) in children are not supportive of its use. Conversely, there are encouraging data regarding the use of CEUS in the setting of paediatric trauma, and in many centres, this imaging modality is routinely used, if not at presentation, certainly as the main follow-up examination [20].

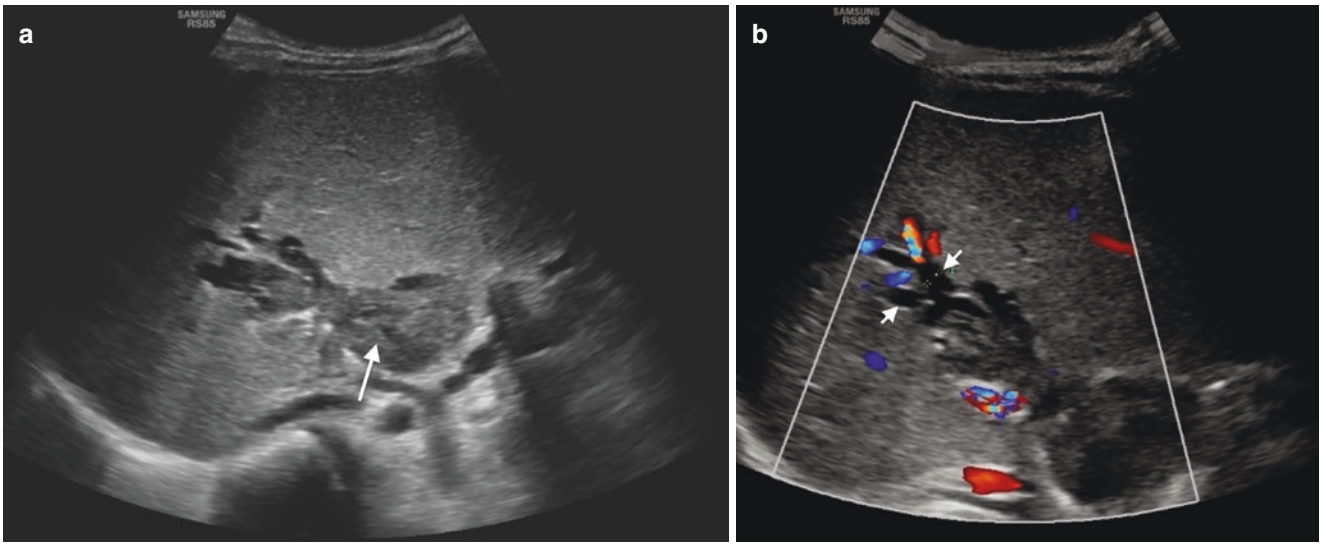


Fig. 57.22 B-mode ultrasound image of a patient with rhabdomyosarcoma relapse demonstrates (**a**) markedly thickened and expanded bile duct (*arrow*). (**b**) Distal intra-hepatic biliary dilatation (*arrows*)

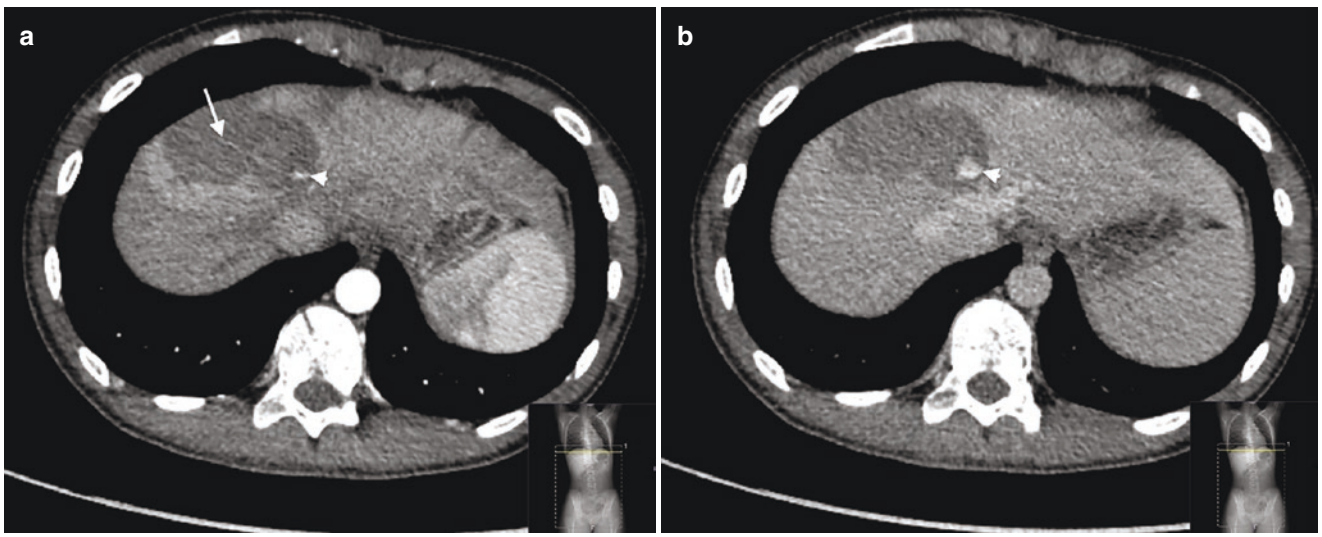


Fig. 57.23 A dual-phase CT scan performed on patient following major trauma shows a large liver contusion (*arrow*, **a**) and active bleeding. (**a**) Extraluminal blush of contrast-enhanced blood in the arterial phase (*arrowhead*). (**b**) Expansion of the hyperattenuating focus on the venous phase (*arrowhead*)

The liver is the second most commonly injured organ in paediatric abdominal trauma (the spleen being the commonest site of injury); the role of the radiologist in this setting is to establish the extent of the injury (in terms of lobes and segments involved and vascular injury) and possible associated complications, such as ongoing haemorrhage (Fig. 57.23). When active bleeding is demonstrated on CT, the patient will proceed to have an angiography with selective embolization of the bleeding vessel (Fig. 57.24). At follow-up, normally between days 5 and 7 of trauma, potentially dangerous complications like pseudoaneurysm of the hepatic artery, hematomas and bile leaks should be actively sought (Fig. 57.25).

CEUS has a higher diagnostic accuracy than conventional US and is comparable to CT for the assessment of liver trauma. It can be used to demonstrate traumatic injuries to the liver, especially at follow-up, to evaluate their extent and complications such as a haematoma, pseudoaneurysm and biloma (Figs. 57.26 and 57.27) [21].

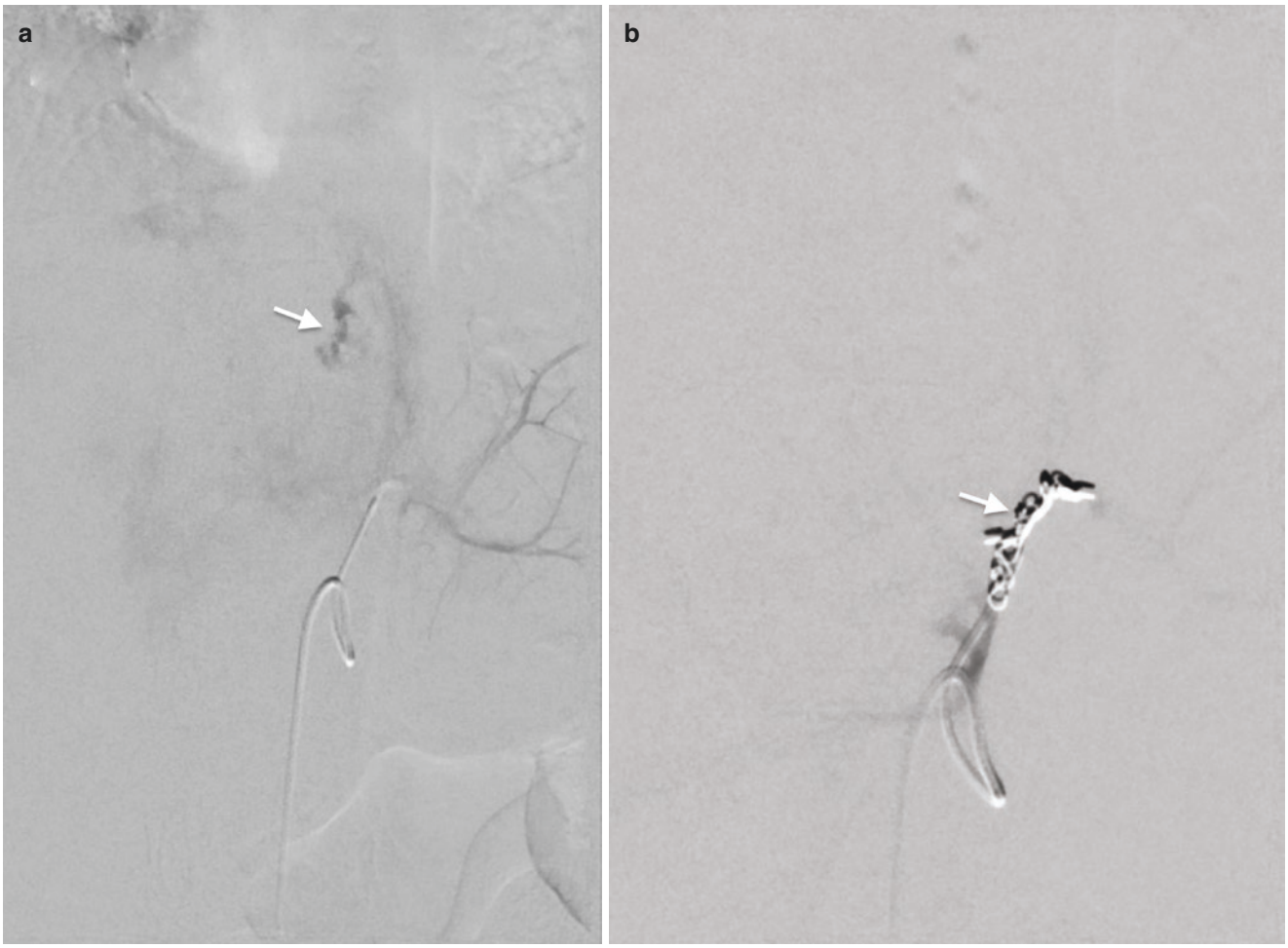


Fig. 57.24 (**a**) Conventional angiography demonstrates selective cannulation of a hepatic artery branch and extraluminal extravasation of contrast (*arrow*) consistent with active bleeding. (**b**) Post-coil embolization (*arrow*) image shows cessation of bleeding

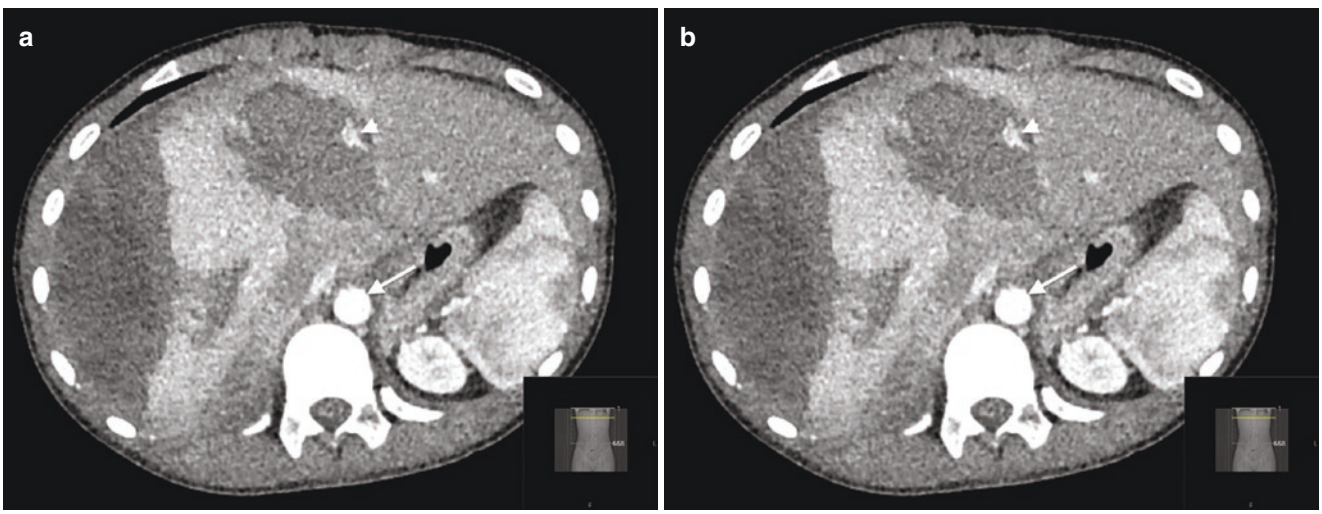


Fig. 57.25 A dual-phase CT scan performed 5 days following a liver trauma demonstrates a pseudoaneurysm. (**a**) Roundish extravasation of contrast-enhanced blood (*arrowhead*) in the arterial phase and has a similar density as the aorta (*arrow*). (**b**) Portal venous phase shows no expansion or change in morphology (*arrowhead*) and has a similar density as the aorta (*arrow*)

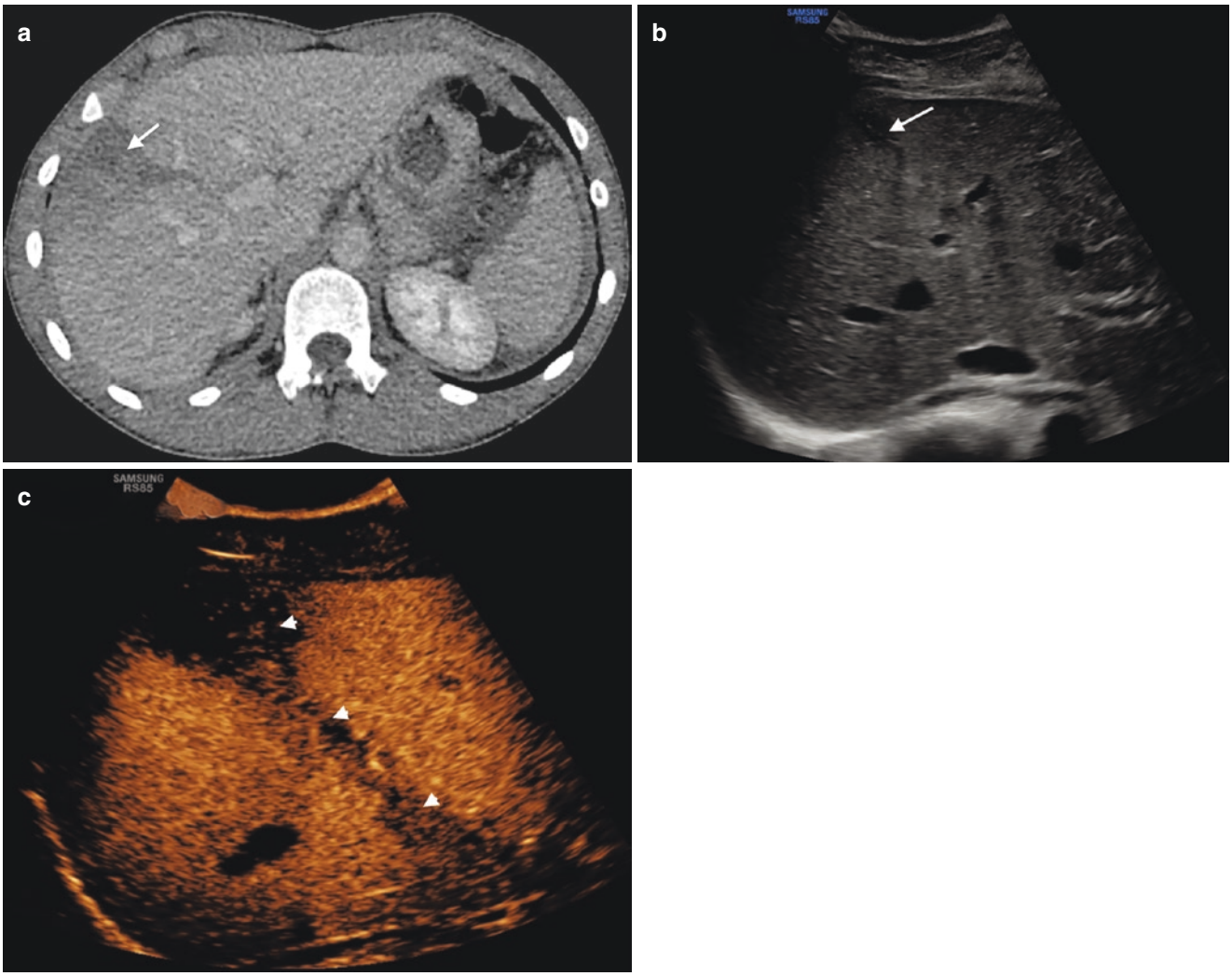


Fig. 57.26 (**a**) Axial CT image of the liver demonstrates a liver contusion/laceration that extends to the hilum (*arrow*). (**b**) Follow-up B-mode image of the liver shows a small focal hypoechoic area in the periphery of the right liver lobe (*arrow*). (**c**) Corresponding CEUS demonstrates the actual extent of the liver contusion and liver laceration (*arrowheads*)

Imaging of Pancreas

Acute and chronic pancreatitis are a significant cause of morbidity and mortality in children; the most common causes of pancreatitis in children are trauma, structural anomalies, multisystem and metabolic disease, drugs and autoimmune disease.

US is the first-line examination of the pancreas and can exclude extra-pancreatic disease; change in pancreatic parenchyma echogenicity is a variable feature, as well as swelling of the gland. US can easily detect dilatation of the pancreatic duct and peripancreatic fatty infiltration, free fluid and collections. In chronic pancreatitis, calcifications can be demonstrated.

MRI with MRCP is the most useful imaging modality to evaluate the severity of pancreatitis and establish further management; in addition, underlying structural anomalies such as abnormal pancreatico-biliary junction and choledochal cysts can also be diagnosed.

Autoimmune pancreatitis shows some specific features: the enlarged pancreas is in fact associated with irregular narrowing of the pancreatic duct, delayed contrast enhancement and a capsule-like rim which is more evident in the early post-contrast images on CT or MR; this corresponds to a fibrosing process extending to the peripancreatic fat.

Pancreatic involvement in children with CF is more frequent than liver disease, with plugging of pancreatic ducts due to inspissated secretions resulting in exocrine insuffi-

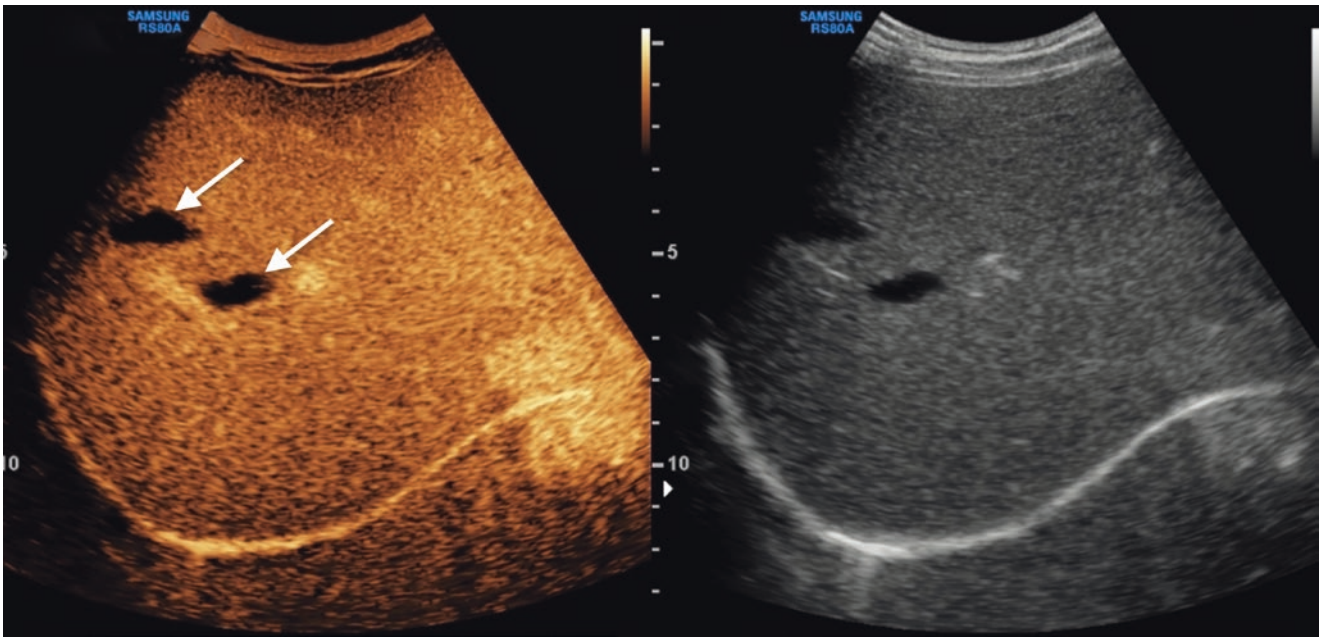


Fig. 57.27 CEUS (left) and B-mode (right) ultrasound images show well-defined anechoic and non-enhanced lesion in the liver parenchyma consistent with post-traumatic bilomas (*arrows*)

ciency. US shows an enlarged or atrophied pancreas with increased echogenicity due to fat deposition; cysts can be present, probably related to the obstruction of ducts.

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Infantile Cholestasis: Approach and Diagnostic Algorithm

58

Narmeen I. Khan and Ruba K. Azzam

Introduction

Clinical jaundice in the first 2 weeks after birth is a common finding, occurring in 2.4–15% of newborns [1]. Often benign, it resolves spontaneously by 2 weeks of age but may persist as long as 3 weeks in a breastfed but otherwise healthy infant. Any jaundice beyond 2 weeks is considered abnormal and requires evaluation with a fractionated serum bilirubin level [2].

Cholestasis, defined as a decrease in bile formation or flow secondary to hepatocellular or obstructive cholestasis, respectively, stems from a variety of etiologies including genetic, metabolic, infectious, and toxin-mediated causes (Table 58.1). Hepatocellular cholestasis is secondary to reduced bile formation, such as due to sepsis or toxins. Obstructive cholestasis is due to either intra- or extrahepatic obstruction, a product of either anatomic or functional obstruction of the biliary system such as with biliary atresia (BA) and Alagille syndrome [3].

Cholestasis is defined as a conjugated/direct hyperbilirubinemia greater than 1 mg/dL if total serum bilirubin is at or below 5 mg/dL or direct bilirubin fraction >20% of total bilirubin when the total is higher than 5 mg/dL. Histologically, bile pigment is present in hepatocytes and bile ducts. Physiologically, the accumulation of substances normally excreted in bile is present in blood and extrahepatic tissues [4].

BA is the most common cause of cholestasis in the neonatal period, accounting for approximately 20–35% of infantile cholestasis [5, 6] and with a wide range of reported incidence rates worldwide. Historically, idiopathic neonatal hepatitis (INH) accounted for the majority of neonatal cholestasis, whereas today, its histologic finding of giant cell transformation is known to be nonspecific and attributable to multiple infectious, metabolic, and genetic disorders [6, 7]. Less than 5% of infantile cholestasis is secondary to congenital infections.

Immaturity of the neonatal liver in bile synthesis, transport, and metabolism renders itself more susceptible to cholestasis. The physiologic development of hepatic function matures in late gestation and over the first few months after birth. This immaturity contributes to a decreased capacity to synthesize and transport bile acids and affects the metabolism, detoxification, and excretion of drugs, xenobiotics, and bile [8–10].

Differential Diagnoses

Conjugated hyperbilirubinemia portends a hepatobiliary pathology that requires further evaluation in a time-sensitive manner. Greater than 95% of neonatal cholestasis cases result from a short list of disorders. Some of these are briefly discussed in this chapter (Table 58.1).

Obstructive

The most common forms of obstructive cholestasis in infancy are BA and choledochal cysts. BA is a fibro-inflammatory disease of the biliary tree. The etiology of BA continues to be researched. It is the most common etiology of chronic cholestasis in infants and children and is the most common indication for pediatric liver transplantation [11]. Affecting 1 in 8000 to 12,000 live births worldwide, there are two major etiologies: perinatal/acquired, accounting for more than 80% of cases, and the rest of BA cases are congenital/embryonic.

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Table 58.1 Causes of infantile cholestasis

Obstructive Biliary atresia Choledochal cyst Syndromic and non-syndromic paucity of interlobular bile ducts Inspissated bile syndrome Congenital hepatic fibrosis (Caroli's disease) Neonatal sclerosing cholangitis	*Infectious* Congenital TORCH infections: Toxoplasmosis Rubella CMV HSV HIV Syphilis Bacterial sepsis Urinary tract infection
	Metabolic/endocrine Bile acid synthesis defects Gestational alloimmune liver disease Neonatal hemochromatosis Galactosemia Tyrosinemia Glycogen storage diseases Hypothyroidism Panhypopituitarism
Toxic Parenteral nutrition Drugs	*Idiopathic neonatal hepatitis*

CMV cytomegalovirus, *HSV* Herpes simplex virus, *HIV* human immunodeficiency virus

Typically, BA presents with jaundice and acholic stools in a well-appearing infant. Physical findings, other than icterus, may not be present in the first few weeks of life especially in the acquired cases. Early detection and timely referral to the pediatric gastroenterologist are crucial since timely surgical intervention with Kasai hepatoportoenterostomy gives the best outcomes if done by experienced surgeons between 45 and 60 days of age [12]. The Roux-en-Y loop of jejunum is anastomosed to the hilum of the liver, creating a conduit for biliary drainage. Patients are usually placed on a high-calorie diet to circumvent malabsorption especially in the case of an unsuccessful surgical outcome, ursodeoxycholic acid, fat-soluble vitamins, as well as antibiotic prophylaxis against ascending cholangitis [11]. Early diagnosis and treatment, in the first 45–60 days of life, improve transplant-free survival to upwards of 75%–90% [13–15]. However, if Kasai fails to clear cholestasis, the 3-year transplant-free survival rate is only 20% [11].

Total bilirubin levels 3 months post-Kasai can indicate prognosis with only 16% 2-year survival with the native liver if the total bilirubin is greater than 6 mg/dL compared to 84% if the total bilirubin level is less than 2 mg/dL [16]. Choledochal cysts are congenital conditions involving cystic dilation of the intra- and/or extrahepatic bile ducts. With type determined by location, these often present with jaundice and are treated surgically within the first year of life. A few cases present beyond this age and into adulthood as well.

Hepatocellular

Hepatocellular cholestasis was historically attributed to idiopathic neonatal hepatitis (INH) which is a prolonged conjugated hyperbilirubinemia with no known etiology. However, in recent years, advanced diagnostic capabilities have made INH a rare diagnosis of exclusion, requiring thorough evaluation for congenitally acquired pathogens (rubella, toxoplasmosis, cytomegalovirus, herpes, human immunodeficiency virus, and syphilis), as well as bacterial infections (gram-negative and gram-positive organisms) as seen in infants with urinary tract infections presenting with jaundice (Table 58.1). Panhypopituitarism should also be considered as a potential cause for unexplained neonatal hepatitis and must be ruled out because a delay in diagnosis may lead to progressive liver disease but also significant endocrinopathies and neurologic disturbances [12]. Once all infectious, genetic, metabolic, and endocrine causes have been ruled out, confirmation of the disease is achieved via a liver biopsy, which demonstrates widespread transformation of multinucleated giant cells, lobular cholestasis in a predominantly canalicular pattern, and hypoplastic bile ducts [17]. The vast majority of these cases resolve spontaneously over a few months [18].

Genetic and Metabolic

Different genetic and metabolic diseases present as neonatal cholestasis, including but not limited to, Alagille syndrome, alpha-1 antitrypsin (α1-AT) deficiency, galactosemia, tyrosinemia, progressive familial intrahepatic cholestasis (PFIC), as well as a variety of other extremely rare metabolic disorders (Table 58.1).

Alagille syndrome is an autosomal dominant multisystemic disease with variable penetrance. It results from mutations of the *Jagged 1* or *NOTCH2* genes responsible for encoding cell surface proteins that interact with Notch receptors during embryogenesis. Presentation is widely variable from being completely asymptomatic to having one or more of the following: chronic cholestasis due to paucity of the interlobular bile ducts, abnormal triangular facies, posterior embryotoxon, butterfly-like vertebral arch defects, and cardiovascular malformations. Patients are also at increased risk for vascular malformations intrahepatically and cerebrovascularly. In infants, prognosis varies with some patients becoming asymptomatic with age, whereas others gradually developing cirrhosis. Supplementation of medium-chain triglycerides, essential fatty acids, and fat-soluble vitamins is emphasized. In extreme cases of cirrhosis, portal hypertension, or severe pruritus, liver transplantation may become indicated [19].

Alpha-1 antitrypsin (α1-AT) deficiency is an autosomal recessive condition that occurs in 1 in 1600 to 2000 live births and is extremely rare in the non-Caucasian population. It results from the intracellular hepatic accumulation of a misfolded α1-AT (a protease inhibitor, PI) protein leading to liver injury [20]. Diagnosis is based on the serum levels of α1-AT and the phenotype, leading to varying degrees of liver, lung (in the form of emphysema), and, less often, skin manifestations. Normal genotype is PI*MM and is associated with normal levels and function of α1-AT. Only those genotypes, such as PI*ZZ, associated with pathologic polymerization of α1-AT within the endoplasmic reticulum of hepatocytes, produce significant liver disease. Neonatal cholestasis is the typical presentation; however, it is highly variable given that only 8–10% of individuals with the PI*ZZ genotype develops any clinically significant liver disease during the first 20 years of life. Two studies indicate that 80% of patients presenting with neonatal cholestasis secondary to α1-ATdeficiency, PI*ZZ, are healthy and free of chronic disease by the age of 18 years [21]. Cirrhosis, portal hypertension, and hepatocellular carcinoma are other hepatic manifestations in older patients.

Yet another autosomal recessive disorder is progressive familial intrahepatic cholestasis (PFIC), a heterogeneous condition associated with the disruption of bile formation [22]. It is divided into at least four subtypes; newer subtypes are being studied further, most of which may have an early presentation in the first year of life. Type I, known as Byler or FIC1 disease, is associated with a mutation in the P-type ATPase FIC gene (*ATP8B1*). FIC1 is expressed in several epithelial tissues including the liver, pancreas, and small intestine. Patients usually present with recurrent cholestasis leading to growth retardation, severe itching, and rickets, among other symptoms, and may progress to cirrhosis if left untreated [23].

FIC1 disease can have extrahepatic manifestations as well, such as recurrent pancreatitis, diarrhea, cough, wheezing, and sensorineural hearing loss. PFIC type II is caused by a mutation in *ABCB11*, the gene coding for the bile salt export pump (BSEP). This defect leads to impaired bile acid transport from the hepatocytes into the bile canaliculi. The gene is liver-specific, and the clinical presentation is similar to that of PFIC type I, though cholestasis is non-relapsing, severe, and progressive. Patients usually suffer from severe pruritus, rickets, growth retardation, and early development of cirrhosis. They are at high risk for early development of hepatocellular carcinoma [23].

PFIC type III results from a gene mutation in *ABCB4* which leads to the defect in MDR3, a member of the ATP-binding cassette family of transporters that serves as a phospholipid flippase. This leads to reduced biliary phospholipid secretion, impairing the balance of the cholesterol saturation index, which leads to crystallization of cholesterol and lithogenicity of bile, ultimately resulting in bile duct injury. Presentation in the neonate is rare, and the spectrum of hepatic manifestations includes cholesterol cholelithiasis, intrahepatic cholestasis of pregnancy, and biliary cirrhosis [23].

Table 58.2 Causes of cholestasis with low-normal gamma-glutamyl transferase (GGT)

PFIC type I
PFIC type II
PFIC type IV
Inborn errors of bile acid synthesis
ARC syndrome
Lymphedema-cholestasis (Aagenaes syndrome)

PFIC progressive familial intrahepatic cholestasis, *ARC* arthrogryposis-renal dysfunction-cholestasis

PFIC type IV is due to a mutation in *TJP2* which encodes for tight junction protein 2 (TJP2). The expression of this protein has been found to be essential to the hepatobiliary integrity, given that canalicular and cholangiocytic membranes are exposed to high concentrations of detergent bile acids [23]. Deficiency in TJP2 leads to leakage of bile components through the paracellular space and into the liver parenchyma, ultimately causing liver injury [23, 24]. PFIC types I, II, and IV are characterized by low to normal serum γ-glutamyl transpeptidase (GGT) levels, unlike type III which presents with high GGT levels (Table 58.2).

Galactosemia and tyrosinemia are two inborn errors of metabolism that result in liver disease. Galactosemia is an autosomal recessive disorder resulting in the inability to digest galactose. It results from deficiency of three different enzymes, the most common and severe type is due to the absence of galactose-1-phosphate uridyltransferase (GALT). Although most states screen for galactosemia, many infants become symptomatic prior to the availability of these results. The typical presentation includes jaundice, lethargy, hypotonia, poor feeding, and hepatomegaly. The diagnosis is confirmed by a deficiency or complete absence of GALT activity in red blood cells. Of note, receipt of a blood transfusion prior to newborn screen may alter results and prevent timely diagnosis. Although dietary restriction of galactose can reverse the hepatic dysfunction, chronic and progressive neurologic impairments and infertility may occur in these patients despite lifelong dietary compliance [3, 12, 25].

Tyrosinemia is an inborn error of metabolism in the tyrosine catabolism pathway, most commonly due to fumarylacetoacetate hydrolase (FAH) deficiency, which results in the accumulation of toxic metabolites such as succinylacetone. It is characterized by acute liver failure and severe renal tubular dysfunction that present in the first weeks or months of life. It can also present as a chronic liver disease. Evaluation includes quantitative measurement of plasma amino acids and urine succinylacetone levels. Dietary

restriction of tyrosine and phenylalanine, as well as early initiation of nitisinone, a potent inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase, is the mainstay of treatment [3, 12].

Lab Evaluation

One must distinguish between direct/conjugated and indirect/unconjugated hyperbilirubinemia in order to properly manage jaundiced infants. The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) published guidelines in 2004 and in 2017 for the evaluation of cholestasis in infants [2, 12]. The group recommended that any infant noted to be jaundiced at 2 weeks of age should be evaluated for cholestasis with measurement of total and direct serum bilirubin. Breastfed infants with an otherwise normal history (no dark urine or light stools) and unremarkable physical examination, however, may be asked to return at 3 weeks of age for further evaluation; if jaundice persists beyond 3 weeks, these infants require further evaluation as well [2]. Multiple studies have supported that early diagnosis, despite the etiology, will lead to better clinical outcomes, especially in BA as discussed earlier.

An algorithm for the evaluation of cholestatic infants is presented in Fig. 58.1.

A thorough history and physical examination can help guide the preliminary steps of the diagnostic process. For example, acholic stools and dark urine are indicators of cholestasis. A study in Taiwan suggested that providing parents with a stool card as a means of "mass screening" was efficacious in early diagnosis of BA [12, 26]. Parents may report a history of easy bleeding or bruising due to a coagulopathy from vitamin K malabsorption and/or liver failure. A complete family history may reveal consanguinity or an inherited genetic disorder. A thorough obstetrical history will identify congenital infections, anatomic abnormalities detected in prenatal ultrasounds, and ABO incompatibility, which, in about 3% of infants, results in intrahepatic cholestasis that resolves spontaneously within a month [27].

Additionally, a complete physical examination may reveal identifying clinical features. Population studies have shown BA to be more common in females of normal birth weight, versus INH, common in low birth weight or premature males. Alagille syndrome may present with both abnormal facies (usually seen beyond the first year of life) and a cardiac murmur. Congenital BA may be associated with structural heart disease, situs inversus, or dextrocardia besides other intra-abdominal anomalies that radiologic evaluation will reveal. Splenomegaly may be found in patients with portal hypertension, storage disorders, or hemolytic diseases [12].

Infants with cholestasis in the setting of sepsis, metabolic diseases, or congenital infections are usually ill-appearing (Fig. 58.1). Congenitally infected newborns are usually small for their gestational age and may have microcephaly, purpuric rash, or chorioretinitis, among other findings.

Confirmation of cholestasis with an elevated fractionated serum bilirubin level requires a concurrent baseline assessment of hepatic function (serum albumin, glucose, prothrombin time/INR, and ammonia) to determine the next step in evaluation (Fig. 58.1). Of note, parenteral administration of vitamin K is recommended in the setting of prolonged INR and should normalize coagulation studies if the synthetic hepatic function is preserved. The alpha-1 antitrypsin (α1-AT) level and phenotype need to be checked early in the diagnostic process, as the histologic differentiation between BA and α1-ATdeficiency is very difficult in neonates (Fig. 58.1). More recently, the serum matrix metalloproteinase-7 (MMP-7) level has been a useful noninvasive tool to distinguish BA from other causes of cholestasis, with sensitivity of 97% and specificity of 83% in one study [28]. The MMP-7 level reflects severity of liver fibrosis and has also been shown to predict the need for liver transplantation in BA patients post-Kasai [28].

Although elevation in alkaline phosphatase (ALP) can be indicative of biliary obstruction, it is non-specific as it is also produced by bone, small bowel, and kidneys. Gamma-glutamyl transpeptidase (GGT) is more sensitive in identifying biliary disease. Low levels of GGT in the setting of cholestasis suggest a short list of hepatobiliary diseases including PFIC types I, II, and IV, arthrogryposis-renal dysfunction-cholestasis syndrome (ARC), and inborn errors of bile acid synthesis (Table 58.2).

Metabolic acidosis on an electrolyte panel or blood gas sample could hint towards a metabolic disease, especially in the setting of other abnormalities like hypoglycemia, hyperammonemia, and lactic acidosis with or without ketonuria. Blood and urine cultures should be drawn when clinically indicated. Septicemia leads to downregulation of biliary transporters, biliary stasis, and hepatic parenchymal injury from circulating endotoxins.

Radiological Evaluation

Ultrasonography is the initial imaging study of choice to assess hepatic size, echogenicity, as well as anatomic anomalies. It also assesses the presence and size of the gallbladder, biliary stones, or sludge. Ultrasound can identify signs of obstruction or dilatation of the biliary tree; extrahepatic anomalies like polysplenia, asplenia, or situs inversus; as well as the different positional variants of the portal vein and hepatic artery. Signs of BA include a small or absent gall-

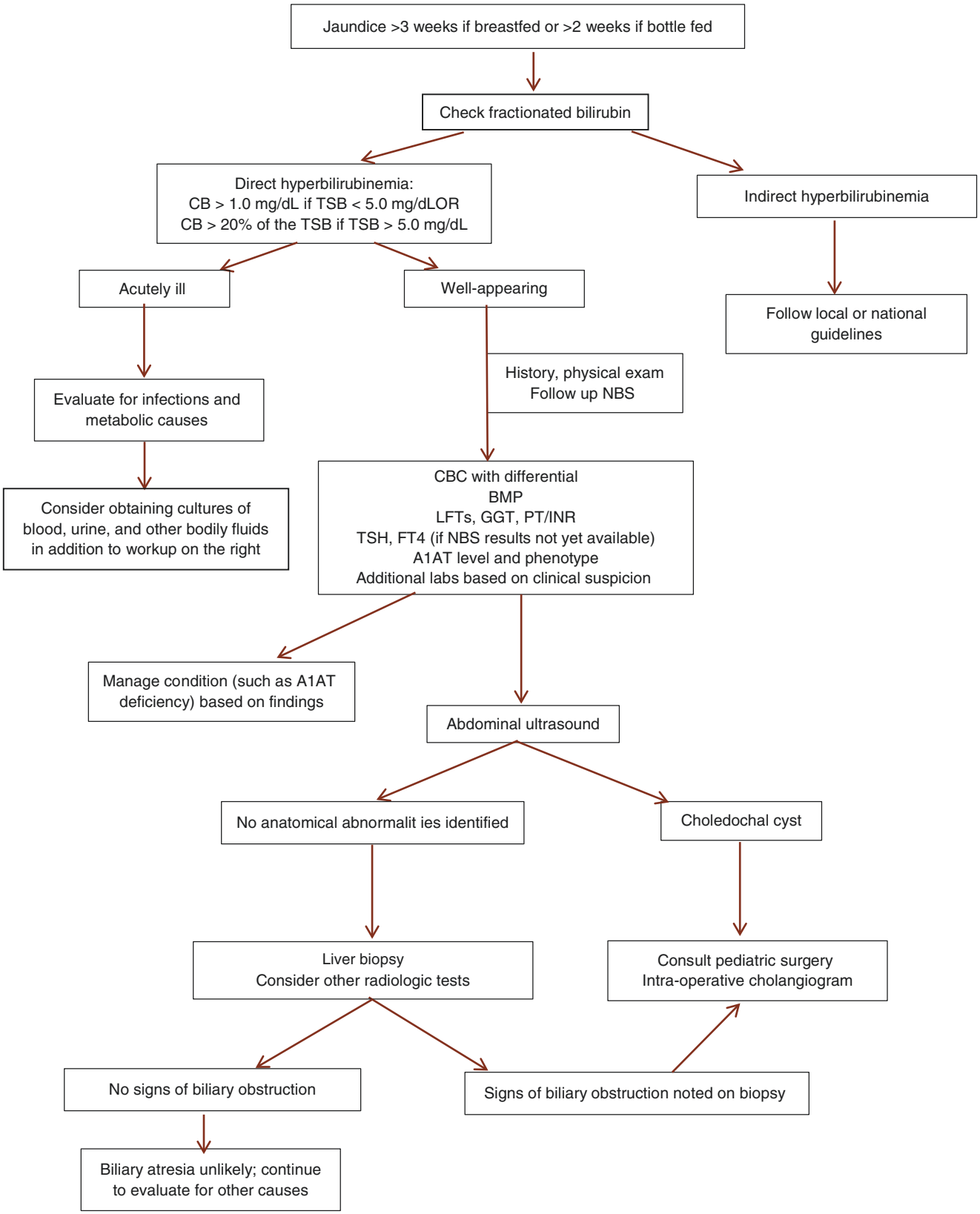

Fig. 58.1 Algorithm for evaluating infants with jaundice

bladder as well as a "triangular cord sign," noted as triangular echogenicity of the anterior wall of the right portal vein on transverse or longitudinal view. Although the use of ultrasound is limited by operator dependence, it is still recommended over computed tomography (CT) which has significant radiation side effects. More recently, transient elastography through elastic shear wave propagation has been found to be a more sensitive and specific noninvasive means to detect severe fibrosis and cirrhosis, such as that seen in BA [29].

Magnetic resonance cholangiography (MRCP), performed with T2-weighted turbo spin-echo sequences, is noninvasive and not operator-dependent but requires sedation. MRCP can evaluate structural anomalies in detail. The addition of gadolinium enhancement for its T2-shortening effect may allow for a more definitive determination of the presence or absence of the duct in infants with cholestasis, especially when conventional MRCP is indeterminate [25].

The ability to differentiate extrahepatic biliary obstruction from non-obstructive causes has been done with hepatobiliary scintigraphy (HIDA) using technetium Tc99m iminodiacetic acid analogues. The addition of phenobarbital for 3–5 days before the test increases its yield but may delay the time to diagnosis.

Endoscopic retrograde cholangiopancreatography (ERCP) is also considered a safe and reliable diagnostic method in cholestatic infants. Its sensitivity and specificity in the diagnosis of extrahepatic causes of cholestasis are superior to other available diagnostic methods when performed by a skilled and experienced endoscopist. In the case of BA and choledochal cysts, the high negative predictive value and specificity of ERCP are superior in diagnosing structural abnormalities of the bile ducts [30].

Following labs and imaging, liver biopsy continues to be an important diagnostic tool, with accuracy reported as high as 90–95% in BA. Timing is important as those performed prematurely will not demonstrate the typical histopathologic features of BA requiring serial investigations and repeat biopsy at 6 weeks of age. Liver biopsy can also be diagnostic of other conditions via electron microscopy, immunohistochemical, or biochemical staining.

The most invasive means of imaging is an intraoperative cholangiogram, the gold standard test to study the patency of the biliary system. Considered the gold standard for diagnosing BA, this study is often elected by surgeons when the diagnosis remains elusive despite imaging studies and liver biopsy [23].

Treatment

Treatment varies widely based upon the cause of cholestasis and will not be explored with further depth in this chapter.

Conclusions

Although the causes of neonatal cholestasis can be many, some of which are benign, prompt and thorough evaluation is indicated to avoid poor outcomes. Fractionated bilirubin must be obtained by 3 weeks of age for infants with prolonged newborn jaundice.

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Biliary Atresia and Choledochal Malformations

59

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Biliary Atresia

Introduction

In 1891, John Thompson, a physician from Edinburgh, described in detail the clinical features and postmortem findings of an infant with what he labelled as "congenital biliary obstruction" [1]. His drawing clearly showed an absence of a common hepatic duct and a collapsed empty gallbladder. Further reports during the early part of the twentieth-century prompted surgeons to explore the biliary tree to try and identify a blockage and perhaps a proximal bile-containing duct to anastomose to. This led to the concept that biliary atresia (BA) was either "correctable" or "uncorrectable" depending on operative findings. With increasing experience it became evident that the latter was much more common and hence survivors were exceptional. Although the use of these terms is nowadays anachronistic (because you can "correct" the uncorrectable!), it perhaps best illustrates the hopeless prognosis of these unfortunate infants.

Figure 59.1 illustrates the various types of BA and is based upon the most proximal level of obstruction. Thus, over 95% are type 3 where there is no visible bile duct in the porta hepatis (hence "uncorrectable"). It does not imply anything about causation (see later).

A more radical approach to the technique was pioneered in Sendai, Japan, during the 1950s and 1960s by Morio Kasai (1922–2008) to the problem of "uncorrectability" [2, 3]. He advocated a more radical approach to the biliary dissection and simply transected at the most proximal point in the porta hepatis. The porta, even if there were no visible ducts, was then anastomosed to a Roux loop (portoenterostomy). Transection exposes residual microscopic bile duct remnants within the fibrous tissue, which retain communication with the intrahepatic duct system (still often very abnormal). Hence bile flow actually occurs to a varying degree, but in perhaps the majority enough to lose their jaundice (see later for results and outcome), postoperative outcome significantly improved.

For the first time, KPE enabled a much larger cohort of long-term survivors, initially in Japan [3] but later from the 1970s in Europe and North America. It wasn't a cure though, and even survivors displayed many complications related to liver fibrosis and cirrhosis.

Thomas Starzl in 1963 attempted the first liver transplant in humans in a child born with BA [4]. Sadly, the child died of operative bleeding related to severe portal hypertension. This prompted a number of units around the world to set up transplantation programmes, but in the absence of effective immunosuppression, they were all terminated by the end of the 1960s. With the discovery of cyclosporine at the beginning of the 1980s, transplantation once more became a viable option, and from this the current strategy of an initial attempt to restore (some might say resurrect) bile flow with a KPE followed by liver transplantation if this fails evolved.

Variants of Biliary Atresia

BA is not a single disease; rather it should be thought of as a phenotype resulting from a number of different and entirely separate aetiologies leading to the final common phenotype of biliary inflammation, luminal obliteration and fibrosis [5].

We have been able to define clinically at least four broad groups (Fig. 59.2).

1. *Syndromic Biliary Atresia*

While BA is usually an isolated abnormality found in otherwise normal term infants, there are a group of infants

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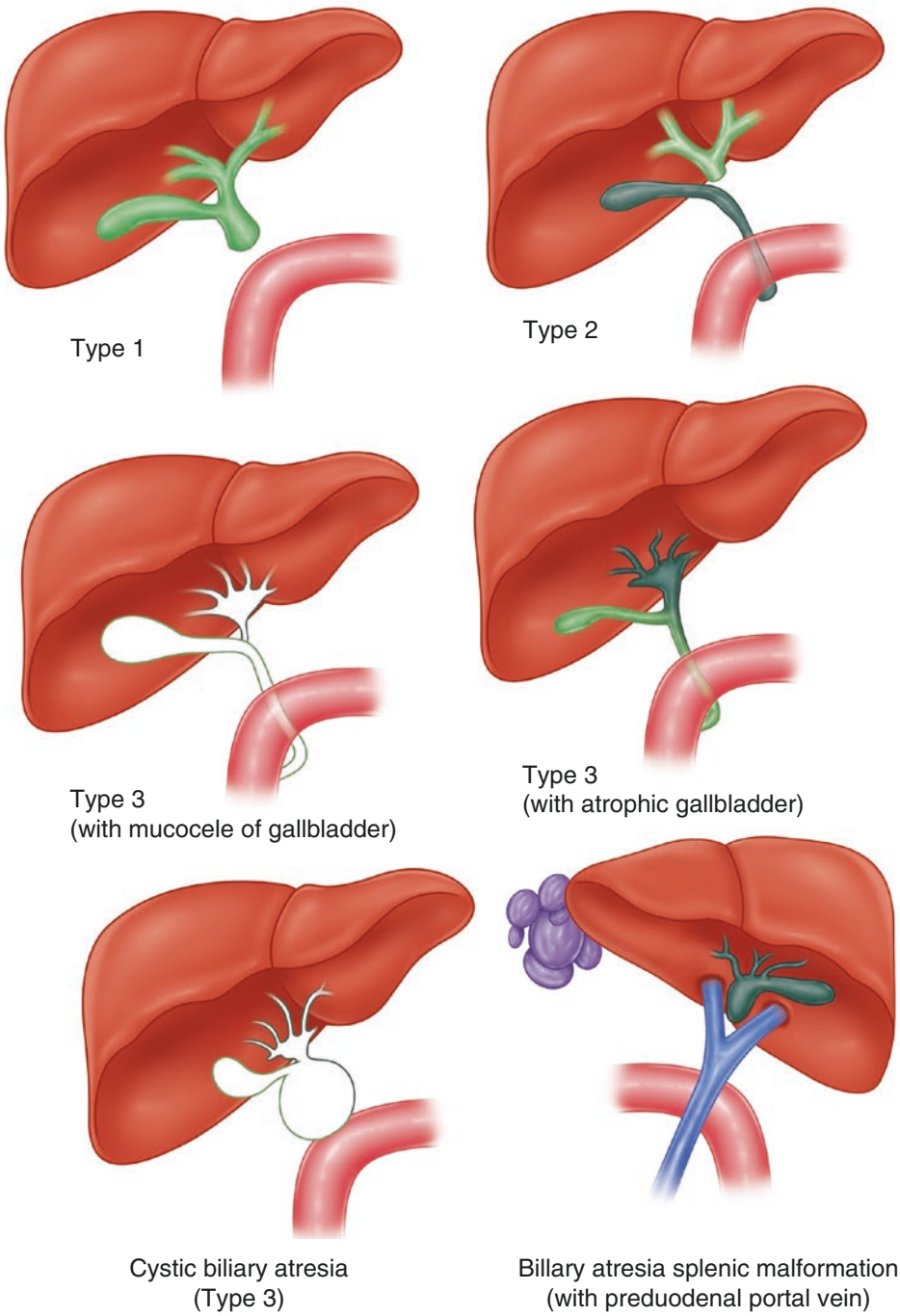

Fig. 59.1 Schematic illustration of biliary atresia. (Based on Japanese Association of Pediatric Surgeons classification)

(about 10–15% in European and North American series, but <2% in Asian series) with other non-biliary anomalies and a poorer prognosis. We have termed this specific constellation of anomalies the biliary atresia with splenic malformation (BASM) syndrome [6, 7]. All will have a splenic malformation, usually polysplenia (~80%) but occasionally asplenia or double spleens, and about half will have situs inversus and congenital heart abnormalities. Other anomalies are evident at laparotomy including preduodenal portal vein, absence of the inferior vena cava and malrotation. Most infants with this syndromic form of BA are female. It is speculated that such cases may result from some fundamental derangement of extrahepatic bile duct (and other systems) development within the embryological phase (<6 weeks of gestation). A large-scale North American study recently identified deleterious mutations in the *PKD1-L-1* gene using whole-exome sequencing in about 10% of subjects with BASM [8]. Other candidate genes have also been suggested such as *CFC-1* and

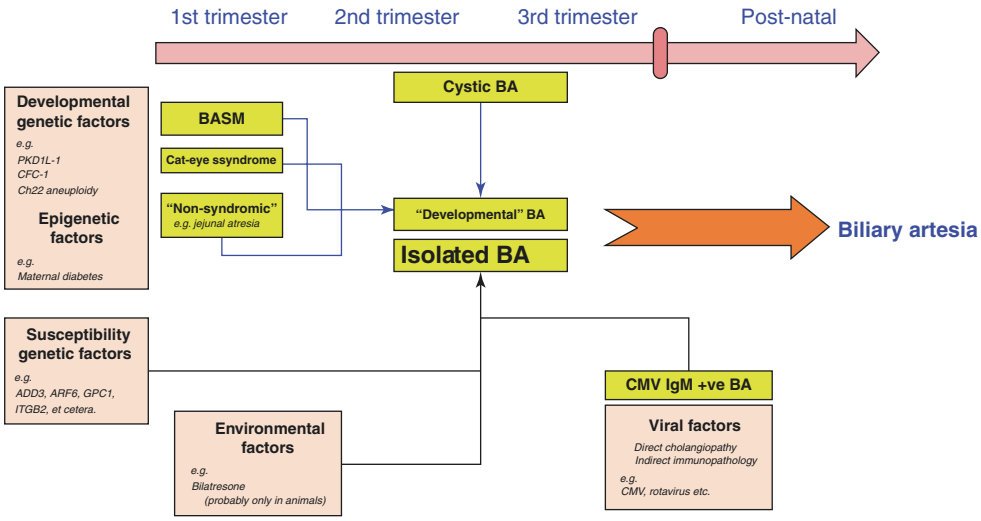

Fig. 59.2 Variants of biliary atresia. Key: Variants ; Proposed aetiological factors

FOXA2 [9]. There is also an association with an abnormal first-trimester intrauterine environment such as that found in maternal diabetes and thyrotoxicosis [7].

2. *Cystic Biliary Atresia (CBA)*

In about 5% of cases, there is an obvious cyst (sometimes containing bile) within an otherwise obliterated biliary tree. In recent years it has been possible to detect this cystic change on antenatal ultrasound scans as early as the 18th week of gestation [10, 11]. CBA should not be confused with cystic choledochal malformation, which can be indistinguishable on ultrasound and MR scans [12]. Discrimination may be made clinically as CBA will invariably have conjugated jaundice, pale stools, etc. and at laparotomy as the cholangiogram will show the abnormal and primitive intrahepatic duct structure.

3. *Isolated Biliary Atresia (IBA)*

This is the typical BA variant accounting for about 80–90% of cases [13]. They have no other significant features and usually display an obliterated biliary tree at laparotomy (usually type 3). Real evidence of what has caused BA in this group is minimal and remains open to speculation. However, some recent evidence towards a developmental pathogenesis was presented by a review of early liver biochemistry (day 1 and day 2 of life) in infants later shown to have BA. This showed that all had elevated direct/conjugated bilirubin by 24 h implying biliary obstruction at the time of birth [14] (Fig. 59.3).

4. *Cytomegalovirus (CMV) IgM+ve Biliary Atresia*

Although there is a range of possible hepatotropic cholangiopathic viruses (e.g. Reovirus type 3, rotavirus), it has been difficult to definitively ascribe clinical consequences to infection. We have recently discriminated infants with CMV IgM+ve antibodies from their IgM−ve fellows clinically, histologically and in their response to treatment [15]. These made up 10% of our clinical series and were more commonly seen in infants from a non-Caucasian ethnicity. Clinically they were older at diagnosis and came to KPE later. Biochemically, they had higher bilirubin and AST levels, with larger spleens as measured on ultrasound than comparable IgM−ve BA infants. The histological appearance within the liver was characterised with an obvious mononuclear cell infiltrate consisting of largely CD4+ Th1+ T cells [16]. These infants also appeared to have the worse response to KPE and also appeared particularly susceptible to death during early childhood [15, 17] (Fig. 59.4). Of all the clinical groups described, this is the group that appears to fulfil most of the requirements to support an immune-destructive pathogenesis following viral triggering.

Epidemiology

Population-based studies reporting incidence and outcomes of BA are scarce. There is marked variation in incidence across the globe ranging from about 1 in 5–10,000 live births in Taiwan [18, 19] and Japan [20] to about 1 in 15–20,000 in mainland Europe [21–24], England and Wales [13] and North America [25].

Furthermore, there is marked geographical variation of the specific variants mentioned above. The incidence of BASM varies widely, being rarely reported in Asian series but accounting for about 10% of European and North American series. By contrast, the incidence of CMV IgM+ve BA also varies from 10% to 20% in European series [15] to perhaps up to 50% reported in some series from China [26].

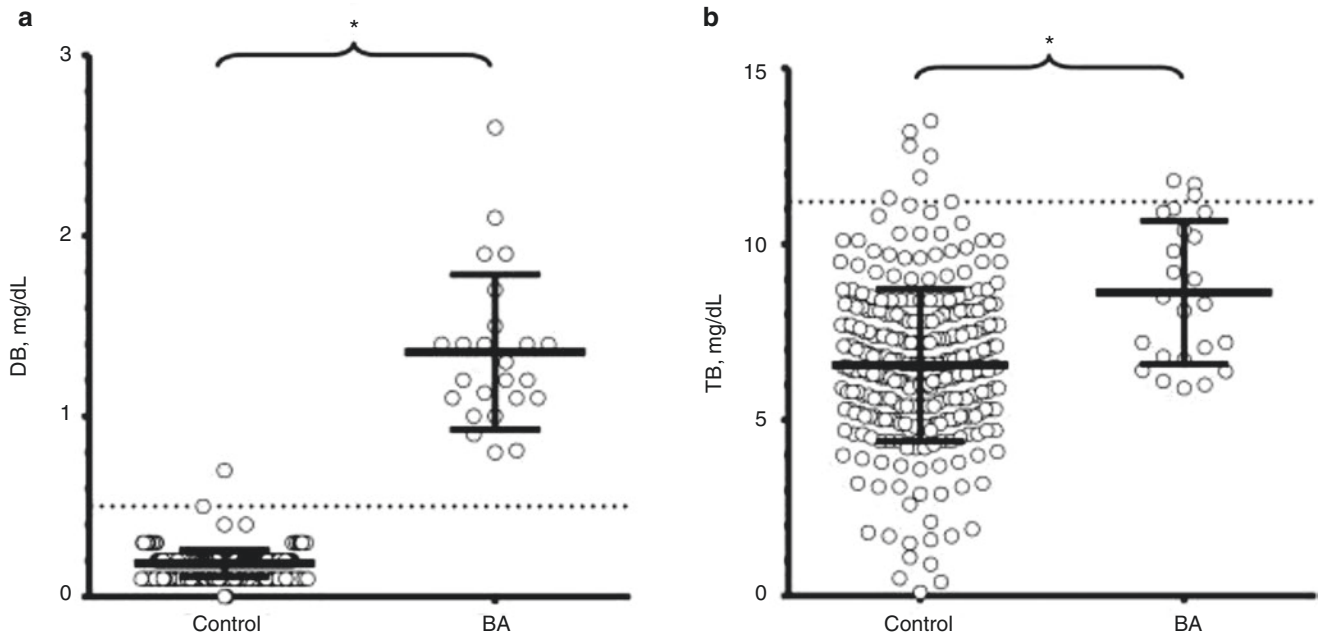

Fig. 59.3 Infants with BA have elevated direct bilirubin (DB), but not total bilirubin (TB), levels at 24–48 h of life (HoL). Shown are the mean DB (**a**) and TB (**b**) levels for controls ($n = 300$; collection time, 39 +/− 5.6 HoL) versus patients with BA ($n = 24$; collection time, 34 +/− 6.2 HoL). The dashed lines indicate the upper limits of normal (**a**, 0.5 mg/dL) or the approximate phototherapy level at 34 HoL (**b**, 11.2 mg/dL). *$P < 0.0001$

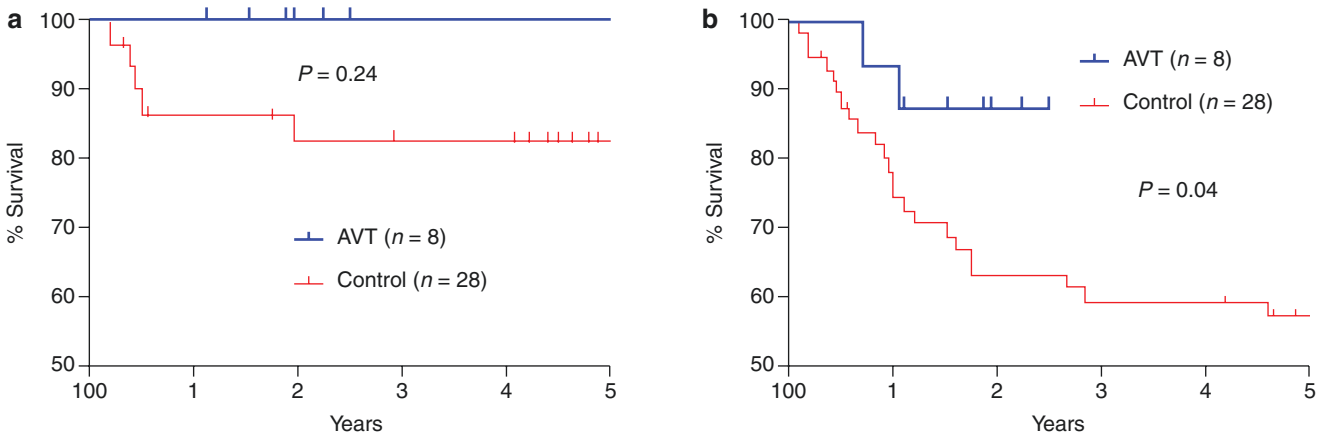

Fig. 59.4 Actuarial native liver survival (**a**) and actuarial survival (**b**) of infants with CMV IgM+ve biliary atresia. NB Both groups (controls and AVT) were treated with Kasai portoenterostomy with (AVT) or without (control) anti-viral therapy (AVT). (Reproduced with permission from Parolini et al. [17])

A number of small series suggested seasonality for BA [18, 27], though these were far from definitive and larger national series failed to observe any predilection for a particular season [13, 20, 21].

Clinical Features

The key features of BA are persistent conjugated jaundice, acholic stools and dark urine in an otherwise healthy term infant (Fig. 59.5). The latter feature is caused by excess conjugated (i.e. water-soluble) bilirubin passing into the urine causing its colour to darken. Such alternative pathways of bilirubin excretion are more developed or at least preserved in the newborn, and very high levels of bilirubin (>300 μmol/L or >17 mg/dL) are exceptional. Sometimes jaundice will be difficult to discern in infants of Asian or Afro-Caribbean origin leading to delays in diagnosis and treatment. The median time to referral was 47 days in white versus 52 days in non-white infants in one UK study [28]. Furthermore, all of those "missed" and referred beyond 100 days were non-white.

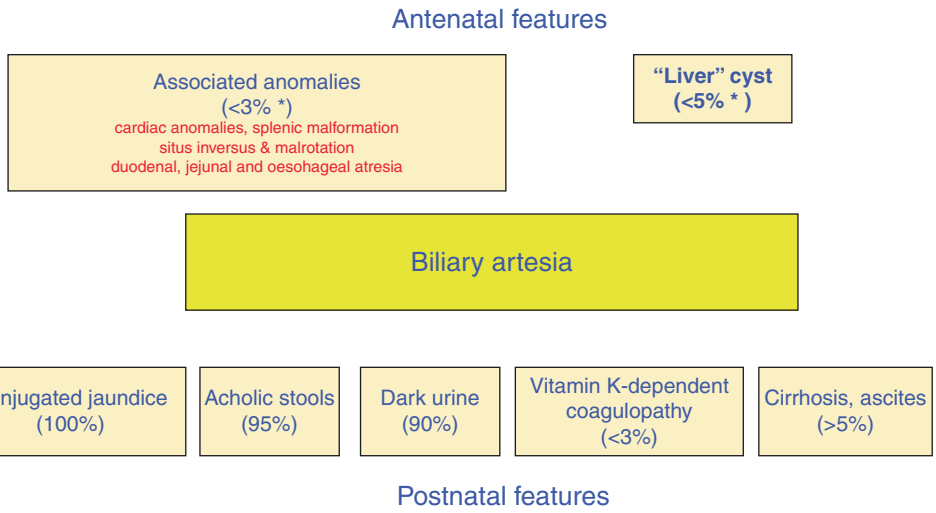

Fig. 59.5 Modes of presentation of biliary atresia. (NB the percentages reflect the mode of presentation not the actual proportion)

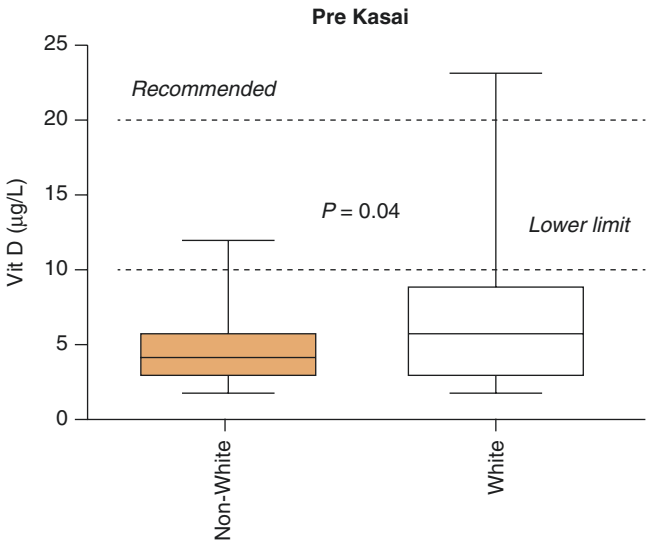

Fig. 59.6 Decreased pre-operative vitamin D levels in non-white compared to white infants with biliary atresia. (Reproduced with permission from Ng et al. [29])

Sometimes the antenatal ultrasound scan will be abnormal showing a sub-hepatic cyst, and one should then be suspicious of CBA [10]. There is no difference in gestational age or birth weight between all the BA variants with all of them presenting with failure to thrive by the time they are admitted. Fat malabsorption is the presumed mechanism, and this will also cause deficiency of the fat-soluble vitamins D, A, E and K. Low vitamin D levels are almost invariable even in those infants presenting early [29], and this is exacerbated in those of Asian family origin, presumably reflecting low maternal stores (Fig. 59.6). Vitamin K deficiency is possible, and a proportion will present with a bleeding tendency, perhaps from the umbilical stump or more catastrophically with an intracranial haemorrhage. Marked elevation of the INR or prothrombin time will be seen in those presenting late. Some syndromic cases will present early (or even antenatally) because of other abnormalities associated with BASM (e.g. cardiac anomalies, malrotation or situs inversus) [30].

Diagnosis

The diagnostic workup in our institution includes a detailed ultrasound of the liver, liver biochemistry and a percutaneous liver biopsy [12]. Using this, more than 90% will have an accurate diagnosis before laparotomy.

Ultrasonography

The ultrasound (US) examination is a key part of the diagnostic protocol as it usually excludes other surgical diagnoses (e.g. choledochal malformation, inspissated bile syndrome, etc.). All of these are being characterised by intrahepatic or common bile duct dilatation. US may be suggestive of BA as a diagnosis – by showing a shrunken, atrophic gallbladder with no evidence of filling between feeds. In about 20% of cases, a "normal gallbladder" is described – these usually turn out to have a mucocele of the gallbladder together with a relatively preserved common bile duct (CBD) and an absent common hepatic duct (CHD) (Fig. 59.1).

Laboratory Findings

Liver biochemistry will show a conjugated jaundice (typically >100 µmol/L), modestly raised transaminases (>100 µmol/L) as well as significantly raised γ-glutamyl transpeptidase (GGT > 200 IU/L). Serum protein and albumin levels should be normal. However, none of these are specific and by themselves are not diagnostic.

Percutaneous Liver Biopsy

In the authors' unit, the pre-laparotomy diagnosis of BA is usually made by percutaneous liver biopsy showing histological features characteristic of "large duct obstruction" such as bile duct proliferation, portal oedema and absence of sinusoidal fibrosis. It is less accurate the younger the infant and it does require an experienced and confident liver pathologist.

Aspartate Aminotransferase-to-Platelet Ratio Index (APRi)

The aspartate aminotransferase-to-platelet ratio index (APRi) can be used as a surrogate of liver fibrosis in many liver diseases, including BA. We have reported that this correlates significantly with age at surgery and was much higher in CMV-IgM+ve BA [31]. Macroscopic cirrhosis evident at laparotomy could also be predicted using a cut-off value of 1.2, with reasonable sensitivity (75%) and specificity (84%) in a large cohort of infants from our unit [31].

The usual differential diagnoses of conjugated jaundice include TORCH infections (e.g. toxoplasma, rubella, CMV, hepatitis, etc.), genetic conditions (e.g. α-1 antitrypsin deficiency, Alagille's syndrome (abnormal "elfin" facies, butterfly vertebrae, pulmonary stenosis), progressive familial intrahepatic cholestasis (PFIC) disorders), metabolic conditions (e.g. cystic fibrosis, galactosemia), parenteral nutrition and neonatal hepatitis.

Miscellaneous Diagnostic Techniques

Some centres rely on more functional tests *looking for* an absence of bile in the intestine such as duodenal intubation and aspiration or hepatobiliary radionuclide scans using a variety of technetium-labelled iminodiacetic acid derivatives (HIDA) [32]. The use of endoscopic retrograde cholangiopancreatography (ERCP) is possible in infants, but its use is typically confined to large specialist centres [33]. Similarly, magnetic resonance cholangiopancreatography (MRCP) is still not detailed enough to confidently diagnose BA.

The "acid test" for many remains operative visualisation (which could be laparoscopic) supplemented by on-table cholangiography where possible.

The surgical differential is less common and includes obstructed choledochal malformation, which usually shows obvious dilated intra- and extrahepatic biliary dilatation, inspissated bile syndrome which usually occurs in the preterm with a precipitating event such as dehydration or haemolysis and spontaneous perforation of the bile duct which usually shows US evidence of bile ascites and a sub-hepatic collection evident on US [12].

Screening

Some countries have adopted a population screening programme for BA [34, 35]. The most well-developed has been that of Taiwan [36, 37], where mothers are issued with colour-coded cards and asked to compare it with their infant's stool. Recognition of pale stool prompts further investigation and referral. This has certainly shortened their time to surgery – the median age at KPE is now <50 days and is currently one of the lowest nationally. The key achievement, we believe, has been the marked reduction in late-presenting infants who had already developed cirrhosis [38].

The simplest solution to early diagnosis remains education however. It is clear from so many histories that it is often the parents who recognise that persisting jaundice in their infants is not normal but then are falsely reassured by health visitors and their community doctors who fail to inquire about pale stools or dark urine (never mind looking at them) and fail to do the appropriate blood test (split bilirubin for conjugated and unconjugated fractions). The statutory community check in the UK occurs at 6 weeks of age and is far too late to make a difference in when these affected infants come to surgery.

Kasai Portoenterostomy

The aim of surgery is to excise all extrahepatic biliary remnants allowing a wide portoenterostomy onto a portal plate, denuded of all such tissue (Fig. 59.7a, b). In most cases, this will expose sufficient transected microscopic bile ductules which retain connections with the primitive intrahepatic bile ductule system to allow restoration of at least a degree of biliary drainage. This should be the object for all three types of BA.

A short right upper quadrant muscle-cutting incision (or laparoscopy) should be performed initially to confirm the suspected diagnosis or, if the gallbladder is shown to contain bile, to proceed to on-table cholangiography.

There are then two surgical strategies to expose the porta hepatis: one involving division of at least the left-sided suspensory ligaments and then eversion of the liver onto the abdominal wall. The other retains the liver in the abdominal cavity but usually requires dissection and slinging of the right and left portal veins to allow full exposure of the biliary remnants. A simple portoenterostomy using a retrocolic Roux loop (about 40 cm) completes the reconstruction (Fig. 59.8). Older techniques involving stomas in the Roux loop have been abandoned by virtually all centres.

Both the dissection and reconstruction in the open KPE can be replicated laparoscopically. However, most centres that pioneered this technique have subsequently reverted to open KPE as outcomes in terms of native liver survival rates and actuarial survival rates are unfavourable compared to conventional surgery [39, 40]. A recent study from Hong Kong confirmed the superiority of the open approach reporting 10-year native liver survival rates of

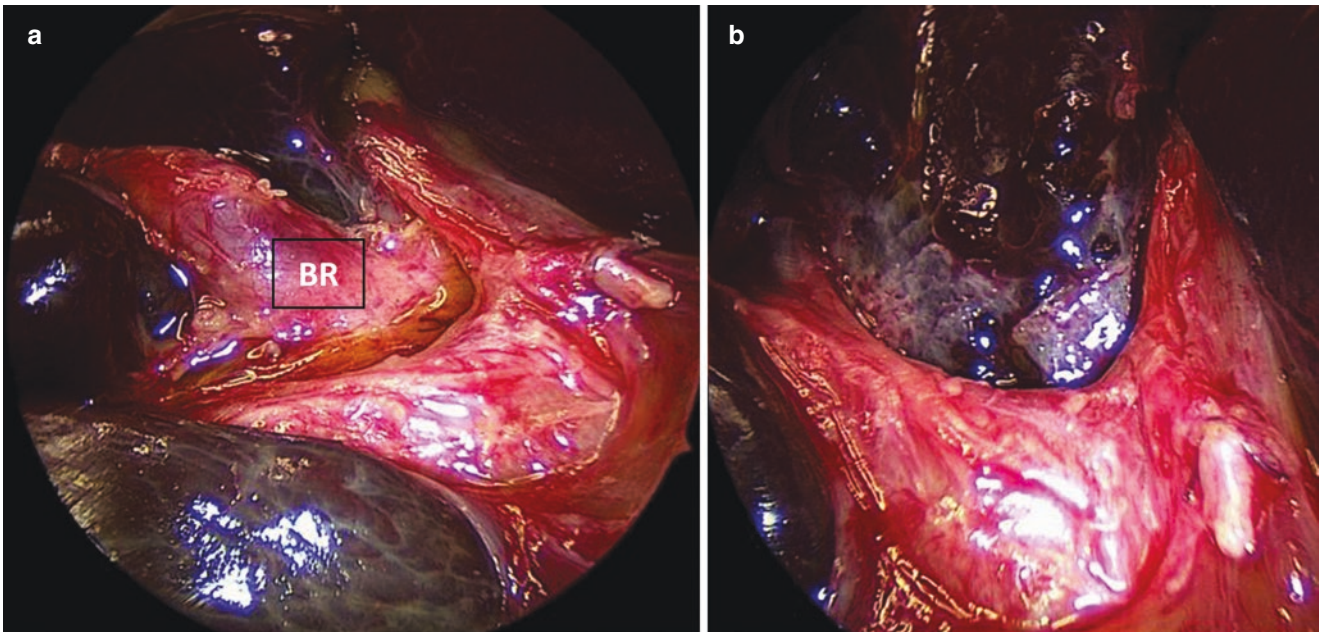

Fig. 59.7 Close-up of porta hepatis during Kasai portoenterostomy. (**a**) Hypertrophied proximal biliary remnant (BR) being separated from vascular structures of liver. (**b**) Same view following resection of BR, showing denuded portal plate

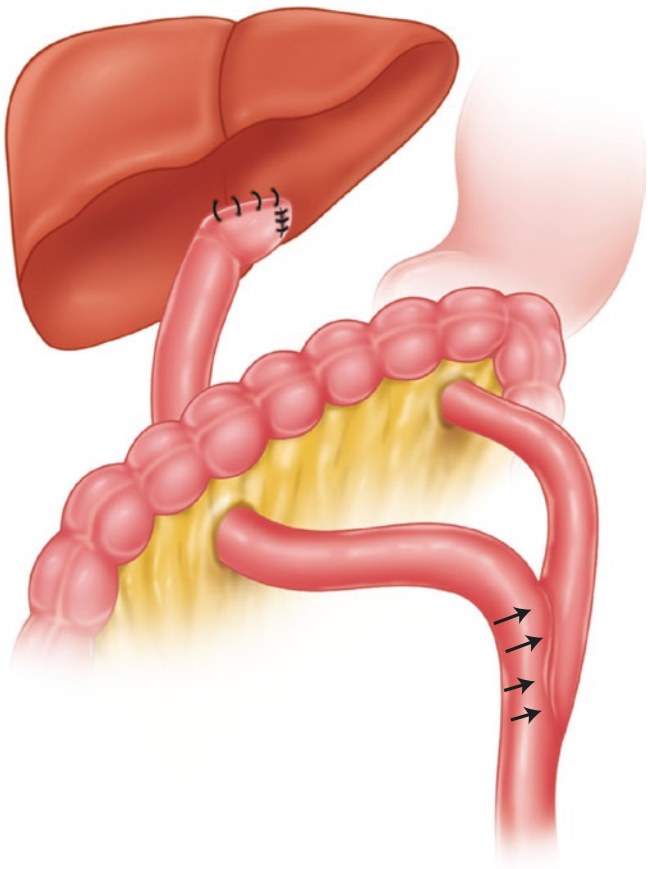

Fig. 59.8 Schematic illustration of retrocolic Roux-en-Y loop, typically measured at 40 cm from portoenterostomy to jejunojejunostomy

85% for the open and 45% for the laparoscopic KPE [41]. The portal dissection, the key to wide excisional surgery, is not improved by being performed laparoscopically, and the reconstruction remains technically challenging. There is obviously a better scar, though the infants remain in hospital for the same length of time and perhaps an adhesion-free abdominal cavity for the transplant surgeon, although even the latter has been challenged by a study from Germany [42].

Surgeons in Juntendo, Tokyo, have adopted a different approach to these issues by limiting the scale of the portal dissection and consciously limiting the transection to a basic oval shape – allowing at least some remnant to remain [43].

Postoperative Management

Intravenous fluids and nasogastric aspiration are continued until return of bowel function (about 3–4 days). Careful monitoring of blood glucose, electrolytes and INR is important in the early phase. Liver biochemistry (including bilirubin) may well worsen in the first week, but by about the fourth week, there should be a definite fall in bilirubin and consistently pigmented stools in those who will do well. Strict attention to nutritional needs is important, and all infants need regular fat-soluble vitamin supplementation. Medium-chain triglyceride (MCT)-based formula milk (e.g. Caprilon®; SHS, Liverpool, UK) is advocated to maximise calorie input and facilitate lipid absorption.

Adjuvant Therapy for Biliary Atresia

As the aetiology of BA remains ill-defined and numbers are relatively few, adjuvant treatment has been largely based on pragmatism and trial and error.

Corticosteroids

Systematic analysis (level 1A evidence) [44] of the few randomised placebo-controlled trial data [45–47] and larger single-centre cohort studies [48] have suggested that postoperative high-dose steroids do have a significant effect on clearance of jaundice with a 10–15% increase in jaundice clearance. This is particularly so in infants <70 days at KPE [49]. We have recently shown that other biochemical markers indicating more specific liver injury (i.e. AST, APRi) are also affected by high-dose steroids at least in the first 6 months post KPE, implying an actual effect on the underlying pathology of the disease process and not just perhaps on degree of restored bile flow [48].

Ursodeoxycholic Acid

The use of oral ursodeoxycholic acid (UDCA) remains popular and may be beneficial but only if surgery has already restored bile flow to a significant degree. Willot et al. from Lille in France assessed the effect of UDCA on liver function in children >1 year post-Kasai portoenterostomy and showed that it improved biochemical liver function in stable children [50]. It may also have an extra beneficial effect in BA because of its immunosuppressive properties as it has been shown to decrease proliferation of and cytokine production by mononuclear cells in vitro [51].

Anti-viral Therapy

CMV IgM+ve BA seems to have an even worse prognosis than CMV IgM−ve BA [15], and for the past few years, we have begun to treat the viral component of this variant. Admittedly this has been on an *ad hoc* basis with variation of the anti-viral agent ranging from IV ganciclovir to oral valganciclovir. However, we have noted a dramatic improvement in the outcome with increased clearance of jaundice and reduction in the medium term for liver transplantation [17] (Fig. 59.4).

Miscellaneous

The benefit of long-term prophylactic antibiotics, bile acid sequestrants (e.g. colestyramine) or probiotics remains unproven. Newer modalities such as immunoglobulin, FXR agonists (e.g. obeticholic acid) and ileal bile acid transporter (IBAT) antagonists (e.g. maralixibat) remain unproven but are now coming into consideration.

Complications

About 20–30% of infants will show no effect from KPE, their stools will remain pale and their bilirubin levels will continue to rise. These infants will inexorably develop cirrhosis and end-stage liver disease with severe failure to thrive, ascites, splenomegaly, deepening jaundice and often bleeding from varices. Such infants need expedited liver transplantation, often before their first birthday. It is important to recognise these infants early and not offer false hope that they will somehow turn a corner – they won't.

Other specific complications deserve a more detailed coverage.

Cholangitis

Re-establishment of bile drainage exposes the child to the risk of ascending cholangitis, which occurs most commonly in the year following primary surgery in about 40–50% of children. Paradoxically, it only occurs in children with some degree of bile flow, not in those with early failure as described above. The usual organisms are enteric in origin (e.g. *Escherichia coli*, *Pseudomonas*, *Klebsiella* spp.).

Clinically, an episode is characterised by fever, acholic stools and a change in biochemical liver function (rising bilirubin and AST levels). The diagnosis may uncommonly be confirmed by blood culture or rarely by percutaneous liver biopsy, but the key component is antibiotic treatment on suspicion not confirmation. Intravenous broad-spectrum antibiotics are effective against Gram-negative organisms (e.g. piperacillin and tazobactam, gentamicin). Most will respond within 24 h, and liver function is usually restored fairly quickly. Some children sustain repeated cholangitis, and they should be treated by prolonged courses of intravenous antibiotics via an indwelling vascular device. If however, it is clear that there are other features of end-stage liver disease, and then they too should be considered for transplant.

Occasionally, cholangitis occurs as a late event in otherwise normal children or adolescents, who have good liver function and have cleared their jaundice [52]. The Roux loop may be at fault here with partial obstruction leading to bile stasis. A combination of radio-isotope scans and percutaneous cholangiography may aid the diagnostic process, and operative Roux loop revision may be required. We have used an enteroscope to investigate these patients and provide radiological and endoscopic visualisation of the proximal Roux loop [53].

Portal Hypertension

Increased portal venous pressure has been shown in about 70% all infants at the time of Kasai operation [54]. However, subsequent portal hypertension depends on both the degree of established fibrosis and, most importantly, the response to surgery. There is a relationship with biochemical liver function and variceal development, and in those who fail and need early transplantation, about 30% will have had a significant variceal bleed.

Infants and children with bleeding oesophageal varices need rapid access to high-quality paediatric facilities with

the resources and expertise to manage them appropriately. Injection or banding is not a technique for the occasional paediatric endoscopist. Restoration of circulating blood volume and pharmacotherapy (e.g. 2 mL/h of 500 μg octreotide in 40 mL of saline) should precede endoscopy and achieve a measure of stabilisation. Sometimes a modified Sengstaken tube needs to be placed to achieve control of bleeding [55]. Invariably in children this can only be done under general anaesthesia but can be life-saving. The definitive treatment in older children is endoscopic variceal banding, although injection sclerotherapy retains a role in treating the varices in infants.

In common with other large centres, we therefore recommend that for each child with BA there is the opportunity to enter a programme of endoscopic surveillance to try and pre-empt variceal bleeding [56]. In this respect, there may be a role for selection based on haematological, biochemical or ultrasound variables to assign risk. One-multinational study suggested that APRi and CPR (clinical prediction rule) [57] appeared to be superior in this respect to simple univariate indices (e.g. platelets, bilirubin) or ultrasound dimensions (e.g. spleen size or resistance index).

The key variceal signs that should prompt *prophylactic* endoscopic treatment are the presence of significant red *wales* in grade II/III oesophageal varices and obvious (usually lesser curve) gastric varices [56]. Liver transplantation needs to be actively considered as definitive treatment for portal hypertension where liver function is poor and the child is already significantly jaundiced.

Ascites

This is related to portal hypertension in part, but there are other contributory factors including hypoalbuminaemia and hyponatraemia. It also predisposes to spontaneous, bacterial peritonitis. Conventional treatment includes a low-salt diet, fluid restriction and the use of diuretics particularly spironolactone. It is often seen in settings of malnutrition and end-stage liver disease, and a nutritional supplementation is important to try and increase calorie and protein intake.

Outcome Following Kasai Portoenterostomy

There are many factors, which will influence surgical outcome in BA. Some are unalterable (e.g. degree of cirrhosis at presentation, absence of, or paucity of bile ductules at the level of section), and some are subject to change (e.g. efficacy of the KPE due to surgical inexperience, poor choice of technique, complications postoperatively due to inexperienced unit, untreated cholangitis, etc.). In large centres with experienced surgeons, about 50–60% of all infants should clear their jaundice and achieve a normal (<20 μmol/L or <1.5 mg/dL) bilirubin [58]. These should do well and have a good quality of long-term survival with their native liver, though the need for transplantation is ever present and the plateau is never flat.

There is no doubt that increasing age at KPE is associated with increasing liver fibrosis and cirrhosis although the actual rates of progression differ according to underlying cause [59]. There is a marked relationship with age at surgery, for instance, with cystic BA and BASM, but it is less evident in isolated BA. So, it is not really possible to use age as a simplistic predictor in individual cases as even in those coming to surgery at >100 days may still have a response to KPE [60]. Still there may be a role for those with the signs of evident cirrhosis (e.g. ascites, heterogeneity of the liver appearance on US) for consideration of transplantation as the primary procedure. This remains an uncommon choice though in the UK and Europe, with <5% of all infants in our series, for instance [58]. By contrast, it seems to becoming a much more prevalent option in certain states in the USA (e.g. California) [61].

In England and Wales, we have adopted a policy of centralisation of surgeons and resources. So for a country of 56 million, there are only three recognised centres to treat this condition, all with surgical facilities including transplantation. This policy was adopted because previous audits of outcome had shown a significant difference depending on centre experience with the less experienced centres showing very poor outcomes [62]. Subsequent studies confirmed an improvement in overall national outcome [58, 63]. Our current outcome statistics show clearance 60–65% of infants coming to KPE at a median now of 48 days. With this at 5 years, we achieve a native liver survival of about 50% and overall survival of 93% (unpublished data; Fig. 59.9a, b). This policy has been successfully replicated by some (typically) Northern European countries [64].

Congenital Choledocal Malformation

Introduction

The German anatomist Abraham Vater recognised the ampullary nature of the junction of biliary and pancreatic ducts which since then has carried his name. Subsequently he also described what appeared to be a choledochal cyst in a pamphlet published in 1723 [65]. Further examples of this, the classical choledochal cyst, were described, but still barely 90 cases had been recorded by 1959, when Alonso-Lej et al. attempted a simple classification into three types [66]. Even as recently as 1975, Flanagan could only identify details of 955 cases from the literature [67].

Choledochal malformation (CM) (of which only some can be described as choledochal "cysts") may be characterised as, … "an abnormal dilatation of the biliary tract, in the

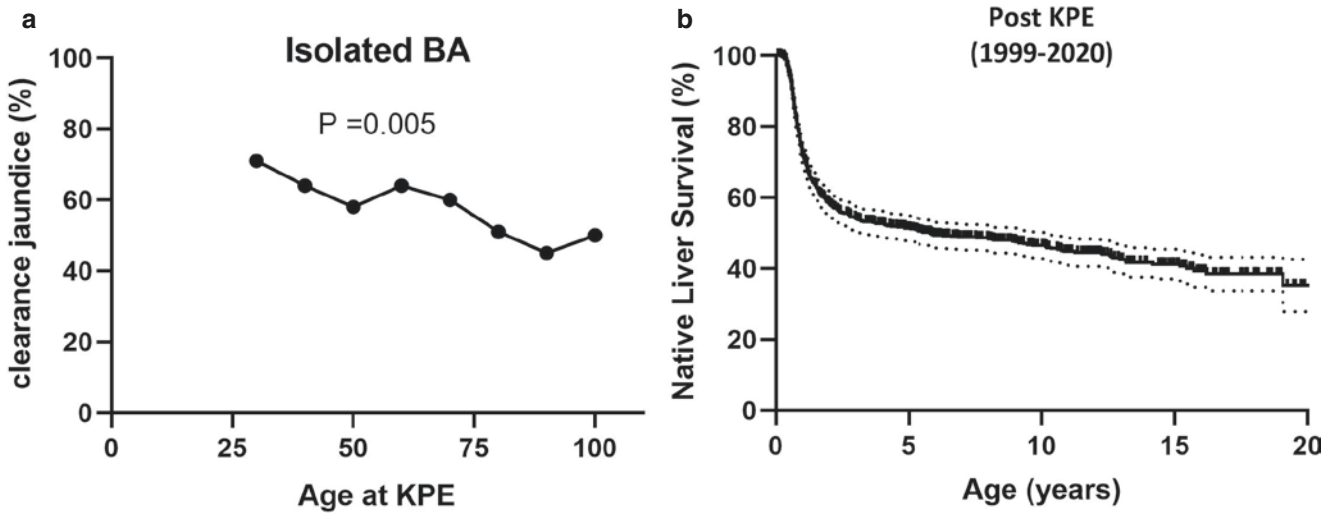

Fig. 59.9 Outcome of biliary atresia in England and Wales since centralisation (1999–2019). (**a**) Age cohort analysis: clearance of jaundice by age at Kasai portoenterostomy. Each point represents individual probability for age-defined cohort (e.g. <30d: 31–40d; 41–50d, etc.). The joining line represents overall influence of age. A "falling line" implies negative influence of clearance on increasing age. Note – NO cut-off! ($n = 613$). (Taken from England and Wales Biliary Atresia database). (**b**) Actuarial native liver survival curve (median (±95% CI)) for biliary atresia ($n = 867$)

absence of any acute obstruction". This allows us to exclude the dilated CBD secondary to choledocholithiasis and strictures secondary to chronic pancreatitis, for instance. Similarly, while many CMs present with jaundice and biliary obstruction, it is obvious that previously their function was unimpaired for much of the subject's life in the presence of clear morphological change.

Aetiology

Most CMs appear in some way to be of congenital origin though the actual mechanism itself is obscure.

There are two competing hypotheses:

1. *Pancreatic Reflux*

An intrinsic part of most examples of CM complex is an abnormal pancreatobiliary junction. Normally the pancreatic and bile ducts open separately within the wall of the duodenum at the ampulla of Vater achieving biological separation of bile and pancreatic juice. In most patients with CM, duct confluence occurs within the head of the pancreas, outside the duodenal wall resulting in a common channel that allows free intraductal mixing of both types of secretion [68, 69].

Donald Babbitt, an American radiologist, proposed that this reflux of presumably activated pancreatic juice could damage the wall of the bile duct causing weakness and then dilatation [70]. There are a number of experimental animal models which have tried to replicate the effects of pancreatic enzymes on bile ducts [71, 72], but there has been little actual documented change in the dimensions of the biliary system.

2. *Distal Bile Duct Stenosis*

Almost 25% of CMs can now be detected antenatally on the maternal US. Most of the infants are not actually jaundiced at birth – though some are and here need to be urgently discriminated from cystic biliary atresia. In all of these, the morphological type is a cystic malformation, and in these, though there might be a common channel, there is minimal amylase in bile (implying no reflux) and often a very definite abrupt change and stenotic distal bile duct segment. Furthermore, animal models involving ligation of the distal bile duct [73] produce obvious cystic change.

To try and resolve some of these questions, we performed a series of studies looking at the relationship between age at presentation, modes of clinical presentation, bile duct pressure (as measured at operation), levels of amylase in the bile (as a marker of reflux) and the histological appearance of the resected choledochus [69, 74, 75]. This showed there is a remarkable inverse relationship between pressure and amylase – the higher the pressure, the lower the amylase and that increasing histological epithelial injury and damage are found in those with higher pressures and very obviously not with high amylase levels (Fig. 59.10).

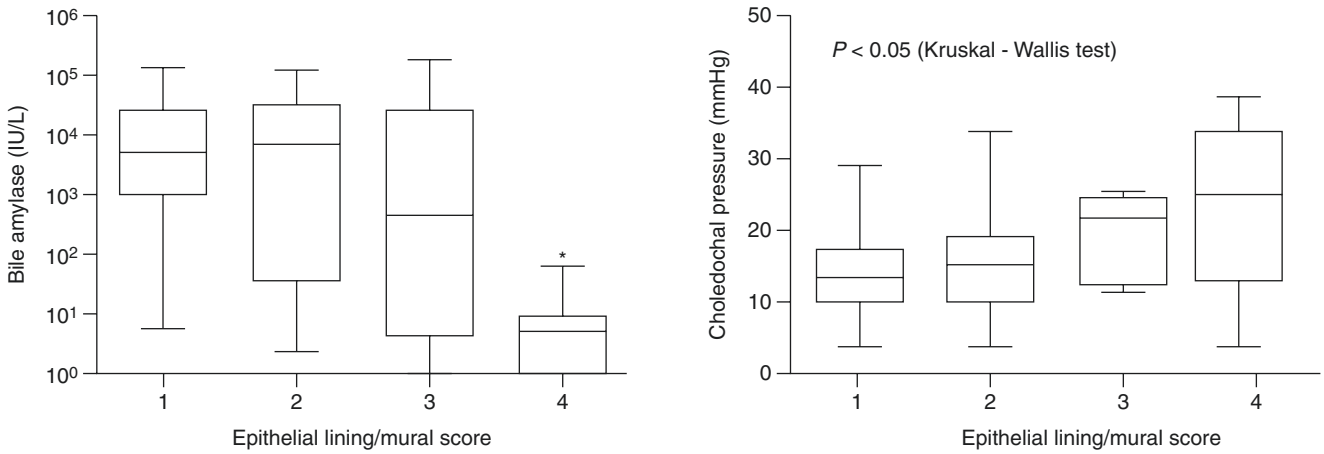

Fig. 59.10 Relationship between biliary amylase, choledochal pressure and epithelial histology. Levels of biliary amylase (**a**) (as a surrogate of pancreatic reflux) and in situ choledochal pressure (**b**) in 73 children with choledochal malformation. The Y axis (epithelial lining/mural score) uses a semiquantitative score for biliary epithelium where 0 = normal, 1 = minimal focal hyperplasia and chronic inflammation, 2 = mild chronic inflammation, 3 = pronounced hyperplasia and moderate chronic inflammation and 4 = epithelial loss, bile impregnation and biliary necrosis. (Extracted from Ref. [59])

Classification

The original Alonso-Lej classification (types 1, 2 and 3) [66] has been modified most notably by Takuji Todani, a Japanese surgeon, by adding the concept of multiple dilatation (type 4) and isolated intrahepatic dilatation (type 5) [76]. The King's College Hospital classification (Fig. 59.11), which has been in use for over 30 years, simplifies the Todani classification into types 1C and 1F (depending on the predominant appearance as cystic or fusiform) and limits type 4 to the combination of intra- and extrahepatic dilatation but again incorporating a cystic or fusiform morphology [77]. This classification has been the basis of our attempts to define pathophysiological characteristics to each type [75, 77–79]. Other workers have emulated this simpler approach.

We also prefer to use the generic term *choledochal malformation*, rather than the very specific term choledochal cyst, since not many of the described dilatations actually appear as a "cystic" (i.e. spherical or globular) entity. The principal variants of extrahepatic dilatation (type 1) making up about 80% of all cases are either *cystic CM (type 1c)* or *fusiform CM (type 1f)*. The former typically has a natural demarcation at either end (Fig. 59.12a), while the latter is much smaller in diameter but merges imperceptibly with a common channel distally or the bifurcation proximally. The only other common variant (*type 4*) is either of the foregoing extrahepatic dilatation (*4c or 4f*) together with significant intrahepatic biliary dilatation – sometimes this is because of actual obstruction with a swift return to normal calibre after surgery, but in others it may appear as an intrinsic feature of the condition (Fig. 59.12b) [79]. Of the remaining variants, *types 2 and 3* are rarely seen in children. The former can be likened to a diverticulum of the CBD, the latter a localised dilatation of the CDB as it transits the wall of the duodenum (a.k.a. choledochocele). *Type 5 CM* refers to usually solitary intrahepatic biliary cystic lesions. Most of these are detected incidentally and can be left alone.

Jacques Caroli, a French gastroenterologist and prolific author, described a number of intrahepatic biliary pathologies which carry his name [80, 81]. The term Caroli disease is usually applied to ectasia or segmental dilatation of the larger intrahepatic ducts (typically the left hepatic duct system) without any other extrahepatic manifestation and is usually sporadic. Caroli syndrome describes a condition in which there are multiple, discrete small yet saccular dilatations of the intrahepatic bile ducts with almost invariably hepatic fibrosis and usually renal disease. This is generally inherited in an autosomal recessive manner [82–84], and there is a large overlap with congenital hepatic fibrosis, polycystic kidney disease (both autosomal dominant and recessive types), etc.

Epidemiology

CMs can present at any point in the life cycle from an antenatal scan to the postmortem table, which makes the true incidence hard to define. If biliary atresia is used as a guide for a condition where the incidence is known (e.g. 1 in 17,000 in the UK) and the ratio of the two conditions presenting in infancy is taken from a specialist hepatobiliary unit, then an approximate figure of about 1 in 100,000 live births may be suggested [85]. Nevertheless, the incidence is much higher in Asian populations, and virtually all of the large series of this has been reported from Japan [86, 87] and now increasingly China [88, 89] with very few series from

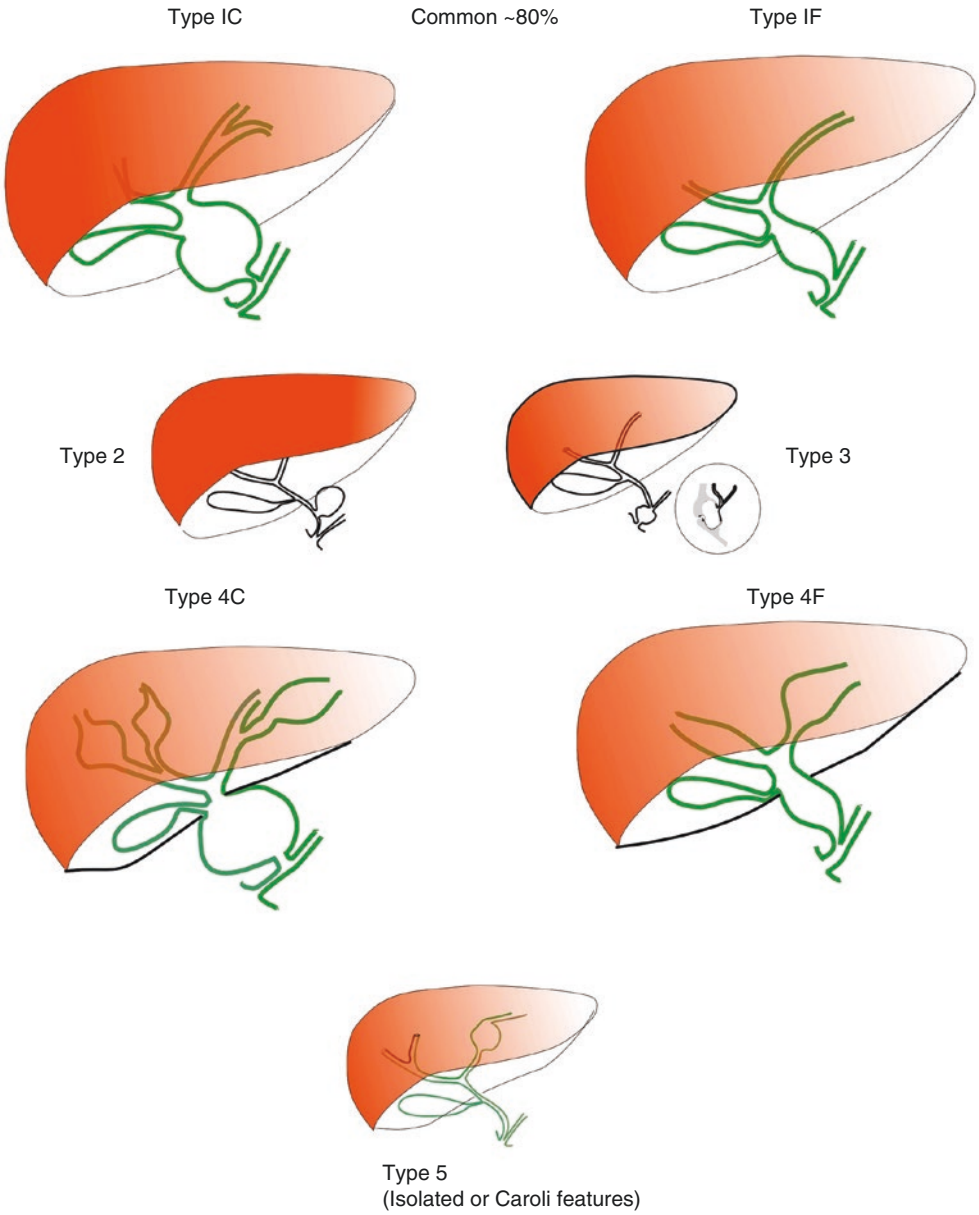

Fig. 59.11 King's College Hospital classification for choledochal malformation

North America [90, 91] or Europe [92]. There is a marked female predominance (4:1) [92], but not much in the way of an identifiable hereditary element. However, isolated examples of familial occurrence in siblings and twins have been reported [93, 94].

Clinical Features

CM can present at any age, but more than 90% will present within the first decade [92]. Clinical manifestations do differ according to the age of onset. Typical presenting symptoms in the newborn mimic biliary atresia and specifically cystic biliary atresia with obstructive jaundice, acholic stools and hepatomegaly, depending on the degree of obstruction. These sometimes have advanced liver fibrosis depending on the length of the period of obstruction. As described above, some will present with an abnormal antenatal ultrasound scan although it will quickly clear their neonatal jaundice.

Older infants and toddlers then tend to present with jaundice and may well be found to have an upper abdominal mass, and most turn out to have a type 1c CM. If obstructive features are ignored, then chronic liver disease may result, and cirrhosis is possible though seemingly not as common as in some older series [92].

Recurrent abdominal pain becomes a feature later on and may be due to an obstructed high-pressure system or actual recurrent pancreatitis. This is usually pathologically mild, oedematous and short-lived and associated with hyperamylasemia. This scenario is usually associated with the type 1f

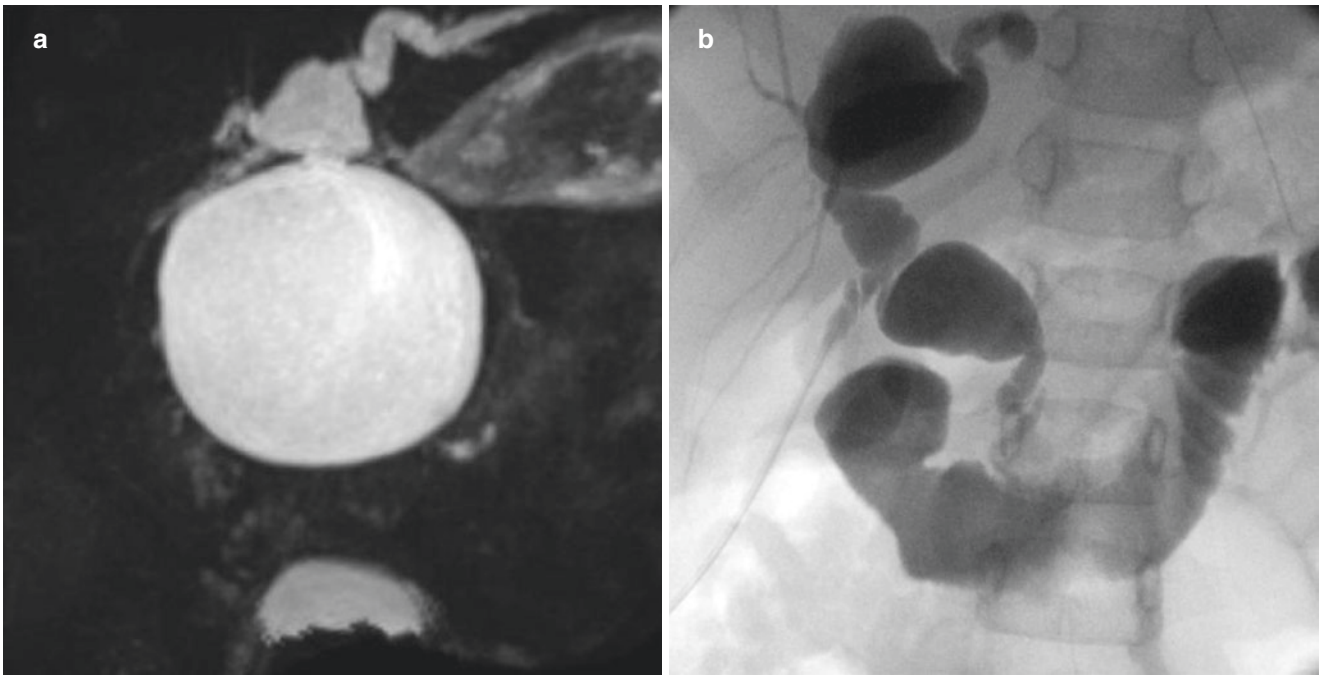

Fig. 59.12 Types of choledochal malformation. (**a**) MR scan (T2 weighted) showing "classical" type 1c choledochal malformation (CM) with a degree of left intrahepatic dilatation. Infant presented antenatally with abnormal maternal ultrasound scan. Note clear demarcation of dilatation both proximally and distally. (**b**) Operative cholangiogram of type 4 choledochal malformation with fusiform extrahepatic appearance and marked segmental dilatation of intrahepatic left hepatic duct. The distal duct gradually tapers towards a common channel (filled with debris) inserting into the third part of the duodenum. A 4-year-old child who presented with acute pancreatitis

CM. Sometimes investigation shows a common channel, presenting features of pancreatitis but not much in the way of biliary dilatation. This has been termed a *forme fruste* CM or more simply common channel syndrome (Fig. 59.13).

Perforation of a high-pressure system is perhaps surprisingly uncommon (<5%), and in these the clinical scenario may mimic spontaneous biliary perforation in infancy [95, 96] or appendicitis in the older child. Bile leakage is usually confined to the retroperitoneum and tracks down the paracolic gutter.

There is a risk of carcinomatous change in long-standing CM which will manifest later in adulthood and exceptionally rarely in adolescence. Up to 10% of adult series have established malignant change at laparotomy [78, 97, 98]. In a Japanese series of 94 patients (adults and children) with excised choledochal malformation, four later developed malignancy. The age at detection ranged from 27 to 65 years with the malignancy arising both in the liver and the head of the pancreas [97]. Similarly, Lee et al. described 80 patients with biliary malignancies from a large Korean multicentre series of 808 adults [98]. Of these most ($n = 74$) were evident at initial presentation and laparotomy while six presented 4 years after their previous choledochal excision. What is not known is what the real long-term risk is in those who have

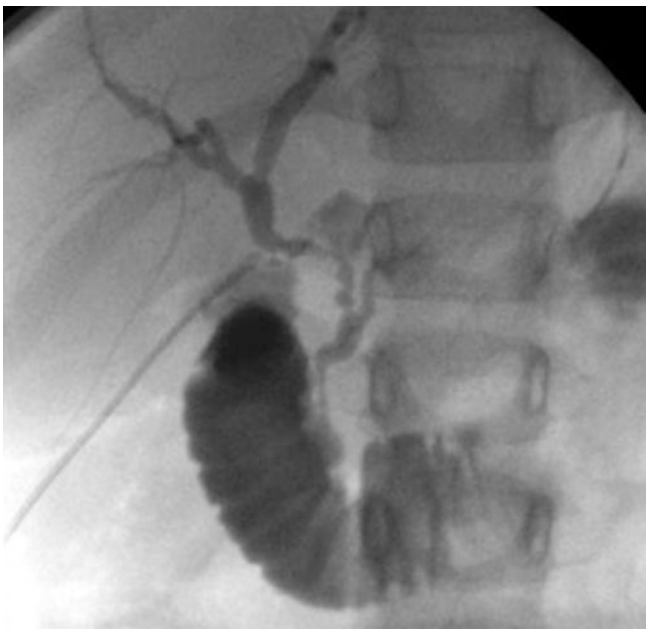

Fig. 59.13 Common channel syndrome. An 8-year-old girl with long history of recurrent acute pancreatitis requiring an ERCP to diagnose a common channel, with presumed reflux of the bile into the pancreatic duct. She was cured by disconnection of biliary and pancreatic ducts and Roux loop hepaticojejunostomy biliary reconstruction

had their definitive excisional surgery in childhood. We tried to identify these on the basis of the histological characteristics of the excised biliary tract (including Ki67 expression), levels of CA19-9 and amylase in bile but there were no consistent features [99].

Diagnosis

A choledochal cyst was first diagnosed antenatally using ultrasound by Dewbury et al. from Southampton in the UK in 1980 [100]. Since then, up to 25% of CM (in the UK at least) are diagnosed antenatally from as early as 15 weeks' gestation, and these are almost invariably types 1c or 5 CM [101]. CM may be confused with duodenal atresia, cystic biliary atresia, ovarian cyst, duplication cyst and mesenteric cyst. In this scenario, it is most important to exclude the possibility of cystic BA who requires urgent Kasai portoenterostomy [11]. If there is clinical doubt, then a dynamic radio-isotope scan (e.g. technetium^{99}-labelled iminodiacetic acid, HIDA scan) will confirm the non-obstructing CM, where surgery can be deferred to about 3–4 months.

Postnatally, US is the initial diagnostic modality of choice, allowing for precise measurements of intra-/extrahepatic duct dilatation and identification of stones/sludge. MRCP has superceded the use of CT and for the most part ERCP for pre-operative anatomical delineation of the pancreaticobiliary tract. Three-dimensional reconstruction images are easily obtained, although sedation may be required in infants and small children.

Functional assessment of CM may be shown using a dynamic radio-isotope scan which can show baseline parenchymal hepatocyte uptake together with the pattern and degree of bile excretion – important if not considering surgery. This may also be useful in diagnosing choledochal perforation. ERCP may still be required when there is diagnostic uncertainty over the nature of the pancreaticobiliary junction and common channel, particularly in those with minimal biliary dilatation and often with a presenting feature of pancreatitis.

Biochemical liver function tests may be normal or show evidence of biliary obstruction. Amylase levels may be elevated during episodes of abdominal pain suggestive of actual pancreatitis. A prolonged INR secondary to cholestasis should be corrected with intravenous vitamin K.

Surgical Management

Open surgery is still very much the standard in most centres. For the common types (type 1c, type 1f and type 4), this consists of excision of the dilated part of the extrahepatic biliary tree, clearance of debris and stones from any dilated intrahepatic ducts (best achieved with on-table cholangioscopy), clearance of any common channel debris (± transduodenal sphincteroplasty) and a reconstruction end-to-side hepaticojejunostomy using a long (40–50 cm) Roux loop. Despite often quite alarming intrahepatic dilatation in type 4 CM, the usual best course is simply excision of the extrahepatic segment with a fastidious approach to ensuring segmental drainage and cautious follow-up. Most ducts will reduce in size, particularly in children which in itself suggests the primary aetiological role of elevated intrabiliary pressure [77, 79].

The first laparoscopic cystectomy and reconstruction of a 6-year-old female with a type 1c malformation was reported by Farello et al. in 1995 from Schio, Italy [102], and has become an option in some parts of the world. The largest series are either from China or Vietnam and now number well over 200 children in each [88, 103]. The technical skills required are high, and a large throughput is important in minimising complications. The standard Roux loop reconstruction is usually carried out by extracting the small bowel through an enlarged umbilical incision though the hepaticojejunostomy is performed intracorporeally. A controversial innovation has been to discard the hepaticojejunostomy because it is difficult and perform a hepaticoduodenostomy which is easier instead [104]. This short cut may be expedient, but the long-term effects of this may be poorer. Cholangitis and biliary gastritis are both significantly more common using this so-called "physiological" alternative [105]. The other alternative, only available in certain centres of course, is robotic surgery which may facilitate the bile duct anastomosis [106], though even here the postoperative stay is no different to those treated more conventionally.

Excision of the diverticulum is probably all that is needed in the rare type 2 CM as long as normal unobstructed distal bile flow can be demonstrated radiologically [107]. Large choledochoceles (type 3 CM) can be removed transduodenally, whereas smaller choledochoceles can be treated by sphincteroplasty or endoscopic sphincterotomy, although admittedly most reported experience is in adults [108].

Most type 5 CMs are isolated, asymptomatic and picked up incidentally with US. They can probably best be treated conservatively with serial US to try and detect any complications such as stone formation [101]. In those with symptomatic or complicated Caroli-like intrahepatic dilatation, resection should be considered particularly if unilobar. The treatment of Caroli's syndrome may be complicated, and occasionally liver transplantation may be considered.

Postoperative Management

Intravenous fluids and nasogastric aspiration are continued until return of bowel function, usually on day 2 or 3 after the operation. Oral feeding is recommenced after the fluid from the nasogastric tube becomes clear.

Complications

Complications are uncommon but may include bleeding, adhesive small bowel obstruction, anastomotic leakage and leakage from the pancreatic duct. Anastomotic leakage may be treated conservatively, particularly if this has followed a difficult and challenging anastomosis. Abdominal drainage is key, and if there is no obstruction to a functioning Roux loop, then it will settle. Pancreatic leaks are less common but more challenging, particularly if the ampullary sphincter is still intact. Consideration should be given to endoscopic ERCP and stenting if a conservative trial of abdominal drainage, intravenous antibiotics, nasogastric decompression and parenteral nutrition fails.

Anastomotic stricture usually followed by recurrent cholangitis and intrahepatic stone formation are late complications [109]. Cholangitis implies a mechanical problem and should be aggressively investigated. Strictures or persistent intrahepatic dilatation can be treated by radiological intervention with surgery reserved for failure. Recurrent pancreatitis implies obstructive problems with the retained common channel. MRCP, but more likely ERCP, should be diagnostic and, in the latter's case, may be therapeutic with endoscopic channel clearing or stenting.

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Congenital Hepatic Fibrosis, Caroli's Disease, and Other Fibrocystic Liver Diseases

60

N. M. Rock, I. Kanavaki, and V. A. McLin

Definitions

Ciliopathies

Ciliopathies are a heterogeneous group of disorders due to cilia abnormalities.

Ciliopathies are multisystemic disorders often sharing common features, such as polydactyly, mental retardation, retinal defects, kidney cysts and fibrocystic liver disease. Multisystemic ciliopathies are described at the end of this chapter.

Fibrocystic Liver Diseases

Congenital hepatic fibrosis (CHF), Caroli's disease (CD), and Caroli's syndrome (CS) are fibrocystic liver diseases and the most common liver manifestations of ciliopathies [1–5]. Their presentation can be only the liver disease or can be associated with a syndrome. Their common feature is the presence of *ductal plate malformation (DPM)*: an abnormal embryonal development of the ductal plate due to impaired ciliary function.

Fibrocystic liver diseases are summarized in Table 60.1. Choledochal cysts may be considered part of the "fibrocystic liver disease" spectrum, because extensive fibrosis and other signs of CHF may be seen on biopsy [6]. The differential of intrahepatic cysts is summarized in Table 60.2 [7–9].

Hepatorenal Fibrocystic Disorders

When the phenotype of the ciliopathy combines only hepatic and renal manifestations, these conditions are also referred to as the *hepatorenal fibrocystic disorders* (HRFCD). The most frequent HRFCD are autosomal recessive polycystic kidney disease (ARPKD) and autosomal dominant polycystic kidney disease (ADPKD).

Pathophysiology

Liver Development Overview

In vertebrates, the early liver bud develops when a few cells from the endoderm delaminate into the adjacent mesenchyme. The early liver cells are called hepatoblasts. They are bipotential precursors which eventually adopt either a hepatocyte fate or a cholangiocyte fate (Fig. 60.1) [10]. As such they express markers of both lineages during development, some of which can be reactivated in certain disease states [11]. Hepatoblasts contribute to intrahepatic biliary development at the ductal plate, a process which is orchestrated by intrahepatic vascular development and which is of importance in understanding fibrocystic liver disease [12].

The Ductal Plate

The ductal plate is the embryonic precursor of the intrahepatic bile ducts. It is first detected by 6 to 7 gestational weeks and consists of a double layer of cuboidal, biliary-type epithelium that surrounds the developing portal veins. This layer develops from the transformation of hepatoblasts and yields an anastomosing tubular network of biliary ducts through a process called ductal plate remodeling (Fig. 60.1) [13–16].

Ductal plate remodeling begins by 11 weeks of gestation and, in normal conditions, is complete in early post-natal life. It is a process of negative selection of non-functional

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Table 60.1 Fibrocystic liver diseases with ductal plate malformation

Disease	Characteristics	Symptoms	Diagnosis	Associated syndromes	Treatment
Congenital hepatic fibrosis (CHF)	Abundant fibrous tissue between portal tracts and ductal plate malformation Rare: latent disease	Portal hypertension: hypersplenism, thrombocytopenia, hepatosplenomegaly Rare: Cholangitis	US Liver biopsy	Ciliopathies	Supportive with management of portal hypertension Porto-systemic shunt Liver transplantation
Caroli's syndrome (CS)	CHF Saccular dilatation of the intrahepatic ducts	Cholangitis Cholestasis, pruritus Abdominal pain Signs of portal hypertension	US MRCP	Ciliopathies Risk of CCA	Antibiotics for cholangitis Liver transplantation
Caroli's disease (CD)	No signs of CHF Saccular or fusiform cystic dilatation to 5 cm of larger and proximal intrahepatic biliary ducts Inheritance unclear but familial cases	Cholestasis Cholangitis No portal hypertension	US MRCP Liver biopsy	Extrahepatic bile duct dilatation Choledochal cyst Risk of CCA	Antibiotics for cholangitis Partial hepatectomy Liver transplantation
Autosomal dominant polycystic liver disease (PCLD)	> 20 round and smooth cysts Non-communicating Development of cyst associated with hormonal features Woman > men **Gene: *SEC63*, *PRKCSH*** **→ *Abnormalities of cAMP pathway*** [1]	Asymptomatic Abdominal mass Pain Cyst infection, bleeding Biliary obstruction No portal hypertension Rare liver failure	US MRCP Liver biopsy	Distinct form associated with ADPKD	Somatostatin analogues (Inhibition of cAMP pathway) Surgical resection Rarely liver transplantation
von Meyenburg complex	Bile duct hamartomas Benign pathology Multiple/unique round uniform cysts 0.1–0.3 cm, Localized close to portal tracts	Asymptomatic, usually found in liver biopsy for other indications Rare portal hypertension	Incidental MRCP Liver biopsy	ADPKD ARPKD Risk of CCA	None
Choledochal cyst	Dilatation of common bile duct +/− intrahepatic and extrahepatic biliary tree Classification based on location Type I-IV: No parenchyma hepatic involvement Type V: Multiple cyst dilatation intrahepatic → Caroli's disease 8–53% cases	Conjugated hyperbilirubinemia Jaundice abdominal pain Acute complication: perforation, bleeding, infection, lithiasis Long-term complication: biliary cirrhosis and hypertension	US MRCP	CHF Risk of CCA	Surgical resection Choledo-enterostomy

CCA cholangiocarcinoma, *US* ultrasonography, *MRCP* magnetic resonance cholangiopancreatography

Table 60.2 Other causes of biliary cysts

Disease	Characteristic	Symptoms	Diagnosis	Treatment
Solitary hepatic cyst, non-communicating	Delimited No communication with biliary duct Right lobe No associated cysts in viscera	Asymptomatic Complications: infection, perforation, obstruction, neoplastic degeneration	Clinical and MRCP Avoid biopsy →infectious risk	Surgery [69]: laparoscopic fenestration or total resection or hepatectomy
Obstructive bile duct dilatation	Stones inside ducts or stricture of ducts from extrabiliary cause (neoplasia; pancreatitis)	Asymptomatic Complication: cholestasis, cholangitis, perforation Signs of primary disease causing obstruction	Clinical MRCP US Signs of primary disease	Surgery Treatment of primary disease

US ultrasonography, *MRCP* magnetic resonance cholangiopancreatography

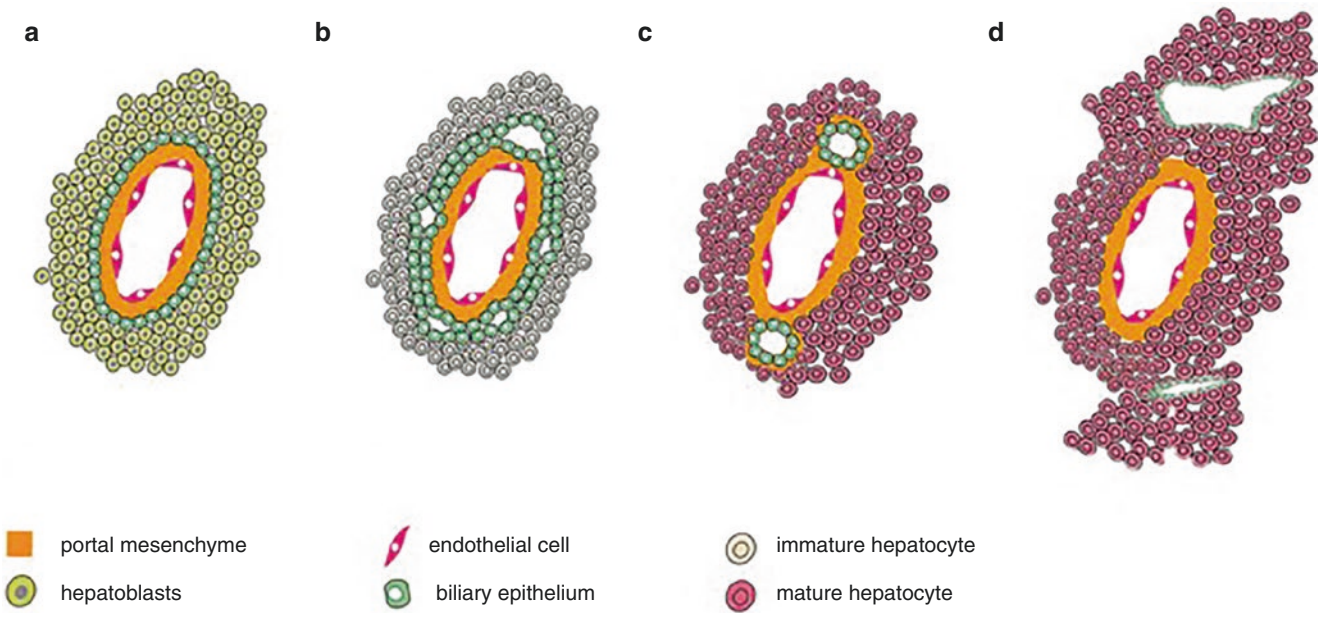

Fig. 60.1 Biliary development and the ductal plate malformation. (**a**) At 14 weeks of gestation, hepatoblasts in contact with portal vein mesenchyme adopt a biliary epithelial fate. (**b**) At 17 weeks of gestation, biliary layer duplicates and focal dilatation within the layer leads to bile ducts formation. (**c**) At birth, bile duct development is nearly completed, and the bilayer has involuted. Liver cells are either hepatocytes or hepatoblasts. (**d**) Ductal plate malformation: abnormal ductal plate remodeling leads to saccular, biliary dilatations. (Modified from [10])

ductal plate elements which involute in a centripetal fashion from the hilum toward the liver periphery [13, 15, 17]. The remodeling phase is characterized by a high rate of mesenchymal proliferation, resulting in the separation of the developing bile ducts from the liver parenchyma (Fig. 60.1) [13, 17]. Meanwhile, hepatocytes and intrahepatic biliary epithelial cells undergo apoptosis at a higher rate than normal under the control of apoptosis-related proteins: C-myc protein and fas antigen stimulate cell death, while Bcl-2 protein inhibits apoptosis [14]. It is possible that an imbalance between proliferation and apoptosis impedes normal ductal plate remodeling, thereby yielding the ductal plate malformation [13, 14]. Remodeling is under the control of a number of genetic and molecular factors that make it vulnerable to maldevelopment. Among these factors are signaling molecules from the portal venous mesenchyme, from adjacent hepatoblasts, from involuting bile ducts, and from hormones such as estrogens [18].

The Ductal Plate Malformation

The term "ductal plate malformation" (DPM) was first used by Mogens Jorgen Jorgensen in 1977 to describe an unusual histologic configuration of the liver of children with polycystic disease. He first described extensive fibrosis and increased number of bile ducts within the fibrotic regions, now recognized as the cardinal features of the DPM [19]. It is now understood that the DPM occurs when certain ductal plate remnants fail to involute. What is not understood is why the size of the bile ducts affected by the DPM varies between diseases: small ducts are affected in CHF, while larger ducts are dysmorphic in Caroli's disease. Figure 60.2 illustrates the DPM and related diseases along a centrifugal axis from the hilum to the subcapsular region. In case of complete lack of remodeling of the ductal plate, the DPM appears like a circular lumen surrounding a central fibrovascular axis. Alternatively, incomplete remodeling results either in a ring of interrupted ducts around a fibrovascular axis or a polypoid projection of biliary epithelium in a dilated duct [20, 21]. Mouse models suggest that DPM occurs as a result of impaired differentiation of biliary precursor cells, failure of maturation of primitive ducts, and defective duct expansion. Interestingly, the same mice models reveal impaired cell polarity and disruption of cholangiocyte ciliogenesis pointing to cilia-mediated cholangiocyte polarity in bile duct (mal)development [22].

There are two groups of diseases in which the DPM is a dominant feature, although it is unclear whether they share a common etiology. The first is characterized by inflammation and destruction of intrahepatic bile ducts with some degree of fibrosis, as seen in biliary atresia. The second is the pattern typically seen in fibrocystic diseases: extensive fibrosis with ectasia and hyperplasia of intrahepatic bile ducts (Fig. 60.2) [16]. Our purpose is to focus on the latter group.

Cavernous Transformation of the Portal Vein

The DPM is often associated with abnormalities in portal vein branching. During normal development, the portal vein

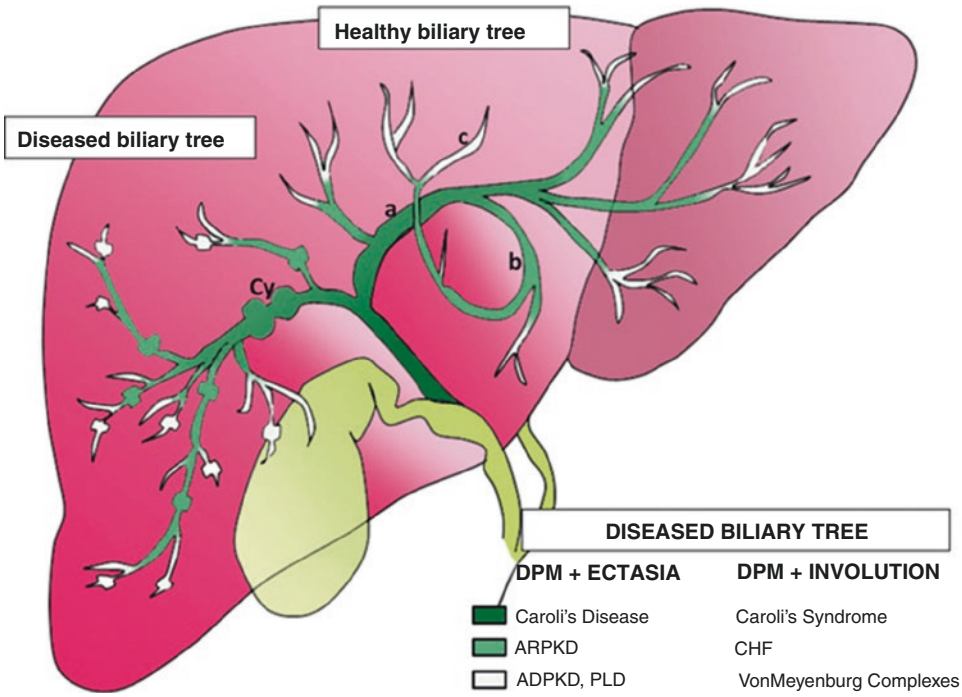

Fig. 60.2 Schematic of intrahepatic biliary tree and level of DPM. For reasons still unknown, defects in ductal plate remodeling impact the biliary tree at different levels. In addition, the remodeling defect yields one of two phenotypes: predominantly fibrotic lesions and dilated biliary structures. Caroli's syndrome is an example of how the two phenotypes might overlap. (Inspired from [20]). *Abbreviations:* Cy kyst. (a) Large biliary ducts. (b) Medium ducts. (c) Small ducts. DPM ductal plate malformation, CHF congenital hepatic fibrosis, ARPKD autosomal recessive polycystic kidney disease, ADPKD autosomal dominant polycystic kidney disease, PLD polycystic liver disease

invades the early liver parenchyma in a centrifugal branching pattern from the hilum to the periphery. This branching pattern determines the lobular architecture of the liver. Therefore, it follows that alterations of vascular development and intrahepatic bile ducts go hand in hand [16, 23, 24]. In the case of DPM, branching is abnormal, thereby leading to the "pollard willow formation," characterized by clusters of unseparated venous tracts [16, 20, 23]. Extrahepatic cavernous transformation of the portal vein occurs in 50–70% of patients with CHF and Caroli's disease and contributes to portal hypertension, one of the main clinical features of CHF patients [23, 24]. Congenital absence of the portal vein has rarely been reported in association with CHF [25–28]. How the extrahepatic portal vein abnormalities are related to the intrahepatic alterations is unclear.

Fibrosis

CHF is a developmental disorder due to DPM of the small, interlobular bile ducts, during which persistence of embryonic remnants stimulates the development of fibrosis. Portal fibrosis is the major histological finding in CHF. The development of fibrosis is a multifactorial process involving hepatic stellate cells, myofibroblasts, and macrophages. Known molecular mediators include transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) [29]. CTGF is known to promote fibrosis [29]. In CHF, CTGF is retained in the extracellular matrix, thereby maintaining a local stimulus for fibrosis. How fibrosis develops in ciliopathies is developed further in the section below on animal models.

Hepatic stellate cells (HSCs) constitute approximately 5–8% of liver cells. They are located in the space of Disse, between sinusoidal endothelial cells and liver parenchymal cells [30, 31]. Activated stellate cells express tissue inhibitors of metalloproteinases 1 and 2 (TIMP 1 and 2) [32–34]. These factors have two roles in fibrogenesis: they exert a direct inhibitory action on metalloproteinases which leads to extracellular matrix accumulation, and they confer anti-apoptotic proliferative signals to activated stellate cells [32, 35]. Finally, stellate cells respond to nearby injury by transforming into myofibroblasts [30] which secrete extracellular matrix (ECM) components leading to scarring [30, 31].

Myofibroblasts are only present in the injured liver and are classically believed to derive from portal fibroblasts or hepatic stellate cells. They are responsible for producing the extracellular matrix which leads to fibrosis [31, 36]. In CHF, parenchymal fibrosis is minimal compared to portal fibrosis. Therefore, it is possible that in CHF, myofibroblasts derive from periductal fibroblasts rather than from parenchymal stellate cells [29].

Finally, *macrophages* have also been recognized as important regulators of liver fibrosis. They are located close to myofibroblasts and interact with the latter and with HSCs [37–40]. They also act independently, by producing their own metalloproteinases and TIMP. They contribute to the phagocytosis of cellular debris and to the secretion of cytokines and immunoregulatory mediators [37, 40, 41].

In summary, portal fibrosis is a major feature of CHF. In response to abnormal ductal plate remodeling, portal fibroblasts transform into myofibroblasts and interact with macrophages which secrete the inflammatory cytokines that trigger fibrosis. At this time, however, the underlying molecular and cellular mechanisms are not clear.

Cilia in Development

Abnormal primary cilia are now understood to be one of the main mechanisms causing DPM. The term *ciliopathies* refers to a heterogeneous group of disorders involving anomalies in primary cilia. Primary cilia are highly conserved organelles used to detect various extracellular stimuli [42]. Although their existence has been known since the nineteenth century, their role in disease is still incompletely understood.

In the liver, cholangiocytes are the only cells with primary cilia [43, 44]. Normal ciliary function is necessary for biliary development. Cholangiocyte cilia are important receptors of mechanical and osmotic signals. They respond by modulating intracellular levels of cAMP and calcium in response to bile flow. This function depends on polycystin 1 and 2 (PC-1 and PC-2) that form a functional complex. PC-1 is a ciliary protein with a large extracellular domain that senses cilia bending. It then transfers this signal to PC-2 which triggers a rise in intracellular calcium which in turn regulates cellular proliferation. PC-1 exerts a regulatory role on growth, such that when its function is impaired or its level of expression diminished, the break is lifted and excess proliferation ensues [45]. Cholangiocyte cilia also detect osmotic changes in the bile via the transient receptor potential cation channel subfamily V member 4 (TRPV4) protein which participates in the regulation of bicarbonate-dependent bile secretion [43, 44, 46–49].

Structural and functional defects of primary cilia result in decreased intracellular calcium and increased cAMP, causing cholangiocyte hyperproliferation and abnormal cell-matrix interactions [43, 47, 50–52]. Estrogens and IGF-1 also contribute to cholangiocyte proliferation, and cystic cells have been shown to overexpress estrogen and IGF-1 receptors. Cell cycle dysregulation also contributes to cholangiocyte proliferation [18, 51].

Excessive cholangiocyte proliferation leads to the development of cysts. Evidence suggests that cAMP signaling dysregulation plays a principal role in cystogenesis. In addition, it appears that the mTOR signaling cascade is involved in cyst growth, since it is upregulated in the epithelium lining the cysts. Finally, cystic cholangiocytes display an abnormal ion transporter pattern leading to increased fluid secretion in the cysts [51, 53–55].

As summarized in Fig. 60.3, cyst expansion in ciliopathies is related to structural and functional defects of the cilia, leading to cholangiocyte proliferation and cyst formation. This process is enhanced by other factors, such as abnormal cell-matrix interactions, cell cycle dysregulation, overexpression of estrogen, and IGF-1 and increased fluid secretion in the cysts.

Evidence suggests that biliary precursors are vulnerable to developmental hits that can impair remodeling. One such scenario is that of somatic mutations occurring during development [56, 57]. A mutation in a single allele of a developmentally key transcription factor such as HNF6 prior to ductal plate formation may lead to impaired ciliogenesis and thus cyst development. Other intracellular mechanisms which can theoretically lead to DPM include loss of heterozygosity, glycosylation defects, and post-translational modification leading to faulty transcription factor binding [58].

Among other developmental steps prone to anomalies, the local growth factor milieu is another variable in a complex web of events. It is probably because the number of genes and signaling pathways are so numerous and the possible combinations of developmental accidents almost infinite that biliary development is so vulnerable to malformations and that there is so much overlap between phenotypes among the ciliopathies and fibrocystic diseases. To help make sense of this wide range of phenotypes, it has been suggested that DPMs can be categorized in one of three ways: perturbations in differentiation of biliary precursors, impaired maturation of radially asymmetrical primitive ductules, and abnormal (excessive) ductular expansion.

Animal Models of Ciliopathies

Much of what is understood today about cystogenesis, fibrosis, and ciliopathies is through animal studies. Animal studies help elucidate mechanisms of pathogenesis and factors of disease progression, identify possible outcomes, and develop treatment strategies.

Although different parameters have been identified leading to cholangiocyte proliferation and cyst development, until recently there was no clear mechanistic studies to

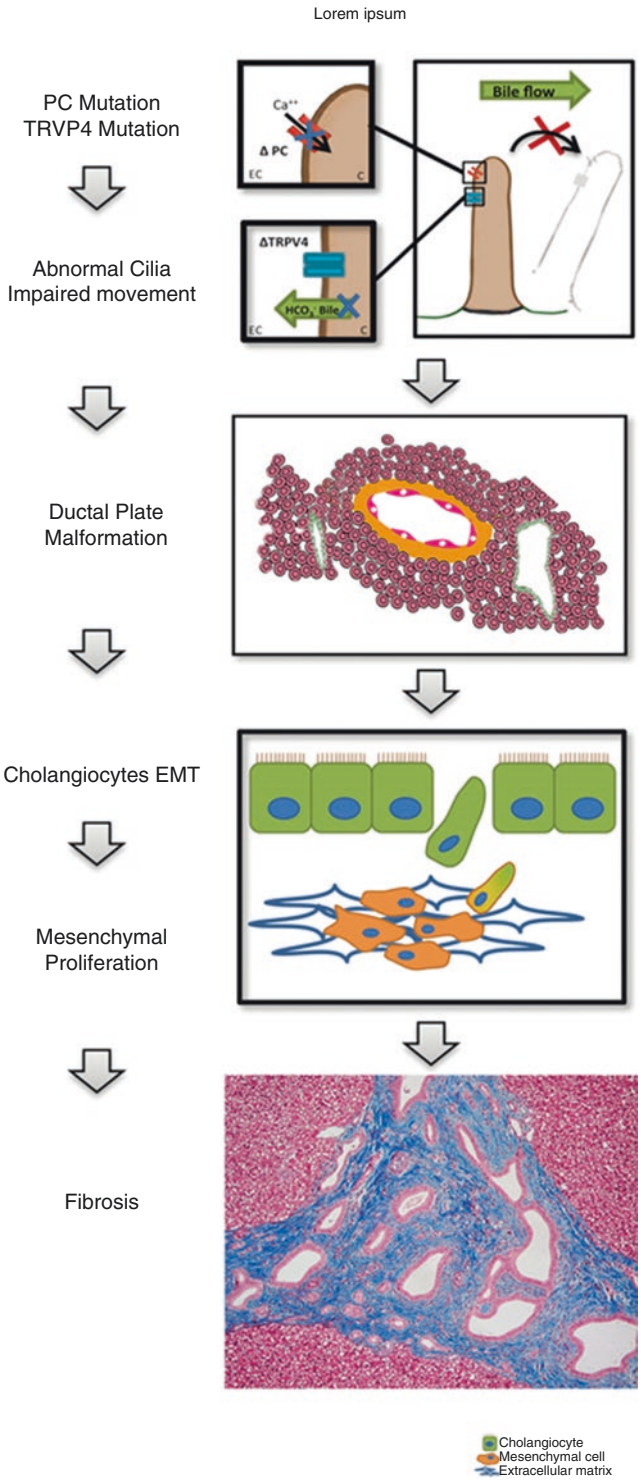

Fig. 60.3 Schematic description of molecular and cellular mechanisms in the hepatic manifestations of ciliopathies. Mutations in polycystin (PC) lead to impaired calcium transport into cilia and thus impaired movement. In case of TRPV4 mutation, osmotic changes in bile cannot be sensed, and a dysregulation of bicarbonate-dependent bile secretion occurs. Structural and functional defects of the cilia lead to ductal plate malformation. Cholangiocytes undergo an epithelial to mesenchymal transition and proliferate in the mesenchyme contributing to fibrosis. *Abbreviations:* PC polycystin, TRPV4 transient receptor potential cation channel subfamily V member 4, EC extracellular, C cytosol

explain the link between cyst formation and fibrosis. Recent studies in rats have shown that abnormal cystic cholangiocytes can undergo an epithelial to mesenchymal transition and secrete extracellular matrix molecules contributing to the development of fibrosis (Fig. 60.3) [59].

The PCK rat carries a splicing mutation in polycystic kidney and hepatic disease 1 gene (Pkhd1), an ortholog of polycystic kidney and hepatic disease 1(PKHD1). Defects in ciliary structure of cholangiocytes lead to abnormalities of biliary tree differentiation and bile duct dilatation, mimicking Caroli's disease. Besides the histological findings described above, macroscopic findings include cholangitis with intestinal metaplasia and hepatic fibrosis [41, 59, 60].

Besides PKHD1 orthologs, other models displaying both polycystic kidney and liver diseases that have include mice with mutations in the genes encoding for cystin, Bicc1, Inversin and Polaris proteins [42].

Animal studies offer insight in the role of signaling pathways in the pathogenesis of liver and kidney ciliopathies. More specifically, receptor tyrosine kinase (RTK) and cyclic AMP (cAMP) pathways are activated in the Cy and PCK rats and in the pcy and jck mice. Furthermore, decreased activation of cAMP pathway seems to improve evolution in PCK and pcy mice. Other signaling pathways explored in rodent models of PCK include intracellular Ca^{2+}, PPAR-γ, MCP-1, and STAT3 that are also targeted in the same rodent models leading to further understanding of disease progression mechanisms in these diseases [61]. Finally, mTOR inhibitions also improve the outcomes of Cy rats, PCK rats, Pkdw $^{WS25/-}$ mice, and Pkd2 $^{flox/-}$ mice.

Congenital Hepatic Fibrosis, Caroli's Syndrome, and Caroli's Disease

CHF, CS, and CD are the most common liver manifestations of ciliopathies in children. Rarely these entities present only with liver involvement, but more often, they are part of syndromic associations which will be described in the next section.

Clinical Features

CHF usually presents between 5 and 7 years of age. Familial cases exist, highlighting the importance of a thorough family history [62–65]. CHF can present in one of four ways [46, 64–66]. First, the most frequent presentation is *portal hypertension* with firm hepatomegaly and splenomegaly [62, 64, 67–69]. Thrombocytopenia and hypersplenism are often present. Occasionally, anemia reveals occult variceal bleeding. The mode of presentation may be an acute gastrointestinal bleed in a previously healthy child. In 30 to 70% of patients that present within the first decade of life, recurrent hematemesis or melena caused by variceal bleeding is frequent, whereas ascites is uncommon [66]. In most patients without an associated syndrome, growth and development are normal. Serum aminotransferase and bilirubin levels are generally within the normal range at diagnosis and remain (near-)normal throughout follow-up. No progression to liver failure has been reported [63]. Complications of portal hypertension such as hepatopulmonary syndrome and portopulmonary hypertension have been described [63, 66].

The second presentation, frequently associated with CD/CS, is *recurrent bacterial cholangitis*. Symptoms include intermittent abdominal pain and pruritus from chronic cholestasis. In this case, serum levels of gamma-glutamyl transferase (GGT), alkaline phosphatase, and bilirubin are elevated. Bacterial cholangitis can progress to septicemia and lead to the development of hepatic abscesses. Recurrent cholangitis may lead to liver failure and failure to thrive in children [66]. Third, patients can present occasionally with *both portal hypertension* and *cholangitis*.

Finally, the disease can be *latent* and revealed by an incidental, clinical, biological, or radiological finding such as hepatomegaly or cytopenias [70–72].

CS/CD can present earlier and later in life. For example, prenatal diagnosis of CS/CD is typically made on the basis of extrahepatic signs of a ciliopathy rather than on the liver alone (Table 60.3) [73–75]. Another mode of presentation is cholangiocarcinoma given the increased risk in the CD/CS population due to bile stasis and chronic inflammation. In the long run, CD and CS are associated with an increased risk of cholangiocarcinoma, thought to be. Cholangiocarcinoma has been reported to occur in 5 to 10% of patients with CD and CS. It has also been reported in patients with isolated CHF, albeit rarely. The average age at appearance is >40 years (range of 33–75 years), and the risk increases with age. It occurs more often in the monolobar form of the disease and has very poor 1-year survival rates [76–79].

Diagnosis

Radiology

Diagnosis should first be suspected on clinical findings. Ultrasonography is a useful, first-line tool for the diagnosis of CHF. Findings are typically heterogeneous liver parenchyma, foci of hyperechogenicity, and images compatible with biliary cysts. The left lobe may appear enlarged and the right lobe atrophic. Splenomegaly and Doppler may be suggestive of portal hypertension and cavernous transformation of the portal vein, although esophagogastroduodenoscopy is the gold standard in the assessment of portal hypertensive complications. Dilatations of the proximal bile ducts and the presence of intrahepatic stones suggest Caroli's syndrome

Table 60.3 Phenotype and epidemiology of ciliopathies. There is no clear genotype-phenotype correlation

Disease	Phenotype												Prevalence	Gene abbreviation	Transmission
	Retinal	Mental retardation	Cerebellar or cerebral	Cardiac	Lung	Pancreas	Liver	Kidney	Polydactyly	Sterility	Situs inversus	Others			
Autosomal recessive polycystic disease							X	X					1:10,000 to 1:40,000	PKHD1, DZIP1L	AR
Autosomal dominant polycystic disease							X	X					1:1000	PKD1, PKD2	AD
Nephronophthisis	X	X		X	X	X	X	X			X		1:100,000	NPHP1, INVS, NPHP3, NPHP4, IQCB1, CEP290,GLIS2, RPGRIP1L, NEK, SDCCAG8, TMEM67, TTC21B, WDR19, ZNF423, CEP164, ANKS6, XPNPEP3, ADAMTS9	AR
Meckel-Gruber syndrome	X		X		X		X	X	X	X	X		1:140,000	MKS1, TMEM67,CEP290, TMEM216, TCTN2	AR
COACH syndrome	X		X				X						<1:1000,000	CC2D2A, TMEM67, RPGRIP1L	AR
Joubert syndrome	X	X	X					X	X	X	X			JBTS1 to JBTS13, ARMC9	
Bardet-Biedl [104, 156]	X	X		X	X		X	X	X	X	X		1:100,000	BBS1 to 19, ARL6, MKKS, TTC8, TRIM32, MKS1, CEP290	AR
Jeune syndrome [106]							X	X	X			Pelvic bone defect, thoracic defect	Rare	IFT80, DYNC2H1, TTC21B, WDR19	AR
Oral facial digital syndrome [107, 141]			X				X	X	X			Cranio-facial defect	1:100,000	OFD1, C2CD3, TMEM107, INTU, KIAA0753, IFT57	XD
Renal-hepatic-pancreatic dysplasia = Ivemark syndrome [108]				X		X	X	X					Rare	GDF1	AR
Ellis-Van Creveld syndrome [109, 155]				X					X			Thoracic defect	Rare Increased in Amish people	LBN, EVC 1, EVC 2	AR
Alstrom syndrome [110]	X			X			X	X					Rare	ALMS1	AR
Leber congenital amaurosis [111]	X	X											Rare	CEP 290, NPHP6, LCA 5, TULP I; REGRIP 1	AR
Senior Løken syndrome [112]	X						X	X			X		Rare	CEP 29, NPH1, NPHP., NPHP4, NPHP5	AR
Mainzer-Saldino syndrome [113]	X		X				X	X				Phalangeal, cone-shaped epiphyses	Rare	IFT140	AR
Carbohydrate-deficient glycoprotein syndrome Ib [114, 115]			X				X					Enteropathy, coagulopathy	Rare	MPI	AR

AD autosomal dominant transmission, *AR*, autosomal recessive transmission, *XD*, X-linked

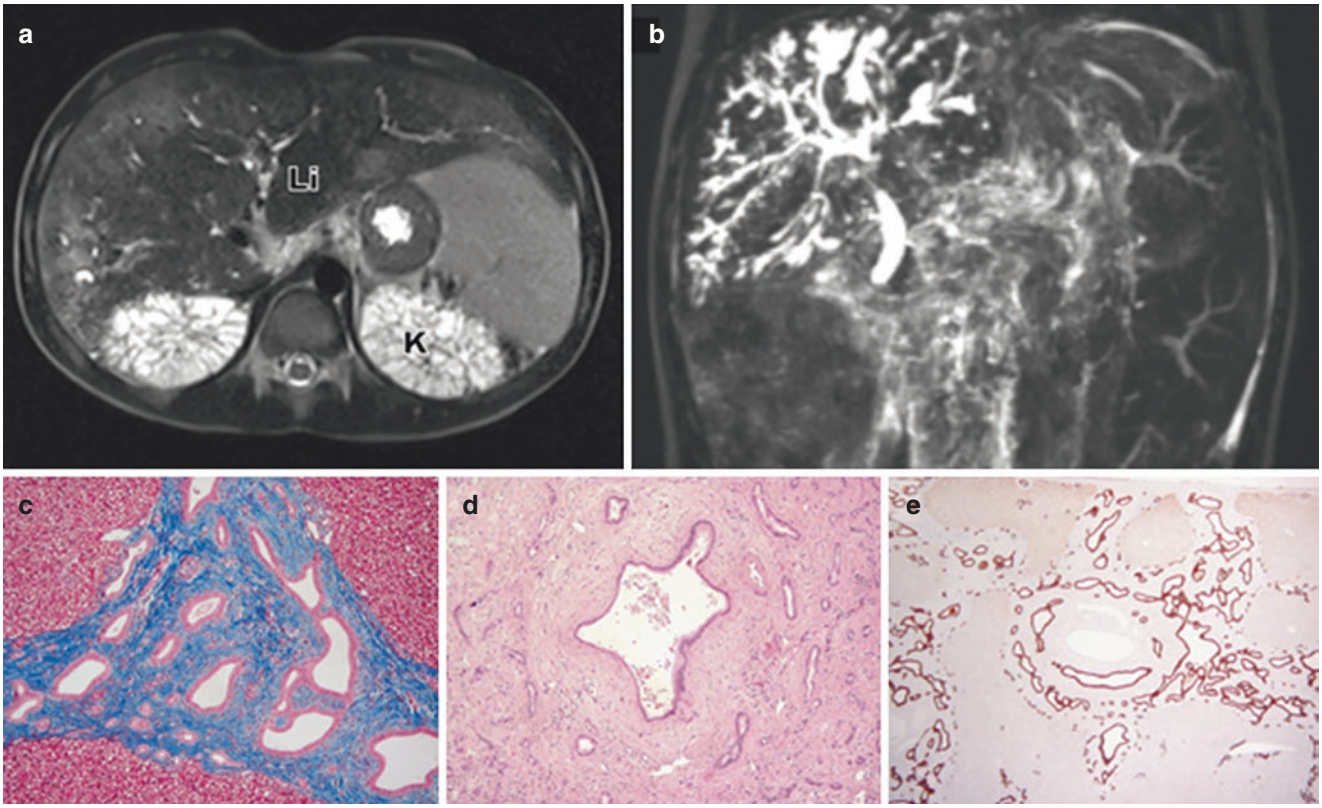

Fig. 60.4 Imaging and histology. (**a**) MR cholangiography illustrating CHF (Li) and polycystic kidney disease (K). (**b**) MR cholangiography in Caroli's disease illustrating intrahepatic biliary cystic dilatation. (**c**) Trichrome staining of a liver biopsy illustrating the fibrosis enclosing portal tracts with adjacent normal lobular architecture. (**d**) Hematoxylin-eosin staining of a ductal plate malformation. (**e**) Characteristic biliary saccular dilatation in Caroli's disease

[65, 66, 80]. In case of ARPKD, kidneys appear hyperechoic on ultrasound with cysts. Since the risk of ERCP-related complications is high, MR cholangiography (MRCP) is best to evaluate biliary disease using 3D reconstructions (Fig. 60.4). Likewise, MR angiography or CT angiography can further characterize vascular findings. US and MR elastography are increasingly used to evaluate fibrosis especially for ARPDK [81–85].

Histology

Liver biopsy is the gold standard to confirm diagnosis, although not always necessary. CHF is characterized by the presence of persistent ductal plate structures of the interlobular bile ducts. Large bands of fibrous tissues link enlarged portal tracts with mild inflammatory cell infiltrates (Fig. 60.4). Within the fibrous bands, branching small interlobar bile ducts lined by normal, cuboidal epithelium are irregularly dilated and may contain bile. The hepatic parenchyma and architecture between portal tracts and centrilobular veins remain normal. There is hypoplasia of portal vein branches and compensatory increased arterial profiles. Caroli's disease is characterized by saccular or fusiform cystic dilatation of larger and proximal, intrahepatic, and extrahepatic ducts without signs of fibrosis. Caroli's syndrome combines the histological features of CHF and Caroli's disease [6, 62, 64, 65, 67, 69, 86, 87].

Management

Treatment of CHF and CD/CS used to be mostly symptomatic and is mainly directed toward the management of complications, such as recurrent episodes of cholangitis and portal hypertension.

The management of portal hypertension is reviewed elsewhere in this volume. Progress has been made toward a pharmacologic approach of patients with polycystic liver disease, aiming to reduce liver volume and to improve quality of life. Long-acting release (LAR) somatostatin analogues such as lanreotide, pasireotide, and octreotide have proven effective in decreasing liver cyst volume in such patients. However, when treatment was interrupted, liver volume increased again. Furthermore, side effects of somatostatin analogues, especially gastrointestinal symptoms, cholelithiasis, and

liver cyst infection, lead to treatment interruption in a number of patients. Ursodeoxycholic acid does not seem to affect liver volume in patients with polycystic liver disease [88–93].

Intrahepatic cholelithiasis is managed with ursodeoxycholic acid and with endoscopic retrograde cholangiopancreatography (ERCP) and sphincterotomy. Partial hepatectomy for recurrent cholelithiasis has yielded satisfactory results [94–96]. In the case of monolobar CD, partial hepatectomy has also been shown to prevent recurrent, life-threatening cholangitis, with acceptable morbidity [76, 77, 97].

Liver Replacement

Liver transplantation is the only definite cure for CHF and CD/CS. It is indicated in refractory portal hypertension, severe growth failure, recurrent cholangitis, decompensated cirrhosis, and liver failure.

The short-term and long-term outcome of liver transplantation in patients with CHF and Caroli's disease is satisfactory overall. Outcomes for CD/CS reach the current average outcomes of liver transplantation for other indications [65, 76, 77, 97–100].

In patients with polycystic kidney disease requiring renal transplantation, combined liver and kidney transplantation yields better results compared to kidney transplantation alone. It has been shown that the outcome of patients with PKD mostly depends on the severity of liver disease and that major complications, such as sepsis, are more frequent in patients with advanced liver disease. Combined liver and kidney transplantation seems to be the treatment of choice for these patients, since nephrotoxic drugs for isolated liver transplantation prior to kidney transplantation can accelerate renal demise [66, 101–103].

Targeting Fibrosis

Although the prospect of antifibrotic agents is attractive and may prove life-saving or at least postpone liver transplantation for some indications, there is no data to show an effect on the fibrosis of fibrocystic diseases. Fibrocystic diseases differ from other secondary causes of fibrosis in several ways: (1) destruction of abnormal cholangiocytes triggers an inflammatory cascade and (2) peribiliary fibroblasts rather than contribute to the extracellular matrix deposition. Therefore, no extrapolation can be made from evidence garnered in other causes of cirrhosis.

Ciliopathies and Associated Syndromes

Ciliopathies with a prevalence greater than 1:150000 are described below, and ciliopathies with low prevalence are outlined in Table 60.3 [104–116]. A practical approach to diagnose liver involvement in case of hepatorenal fibrocystic disease is suggested, and the crucial role of genetic testing is highlighted at the end of this section.

Autosomal Recessive Polycystic Kidney Disease (ARPKD)

ARPKD is the most frequent ciliopathy in childhood, affecting in 1:20,000 live births. It is characterized by progressive cystic degeneration of the kidneys and CHF. As its name suggests, transmission is recessive [108, 111]. Eighty percent of patients have mutation in polycystic kidney and hepatic disease 1 (PKHD1) gene. For the rest, a variation of PKHD1 genes (increase of the number of copies or second locus mutations) have been identified. DAZ-interacting zinc finger protein 1-like (DZIP1L) mutation has been reported in moderate ARPKD [112, 114].

Presentation All patients diagnosed with ARPKD have CHF from birth on microscopic examination, but only 50% have hepatomegaly. CD and CS were also described in association with ARPKD. Most of the patients are born with nephromegaly from dilated collecting ducts which can present as a palpable flank mass. Thirty percent of patients die at birth from pulmonary hypoplasia (Potter's syndrome). There is a marked variability of phenotypes even among siblings [113].

Renal Disease The course and the severity of renal disease and liver disease are unrelated. In the infant or neonate, presentation is usually renal, while CHF is typically asymptomatic early in life. Renal disease can present as a simple urinary concentration defect or more often as severe cystic kidney disease leading to hypertension and renal failure. End-stage renal disease affects about 30% of children under 10 years. For neonates surviving the first month of life, the 5-year survival rate is about 90%. Most patients presenting as neonates will require kidney transplantation before 20 years of age.

Liver Disease Improvement in the management of renal complications has led to an increased awareness of the liver-related morbidity and mortality due to CHF. In addition to liver and kidney disease, intracranial and splenic aneurysms have been

described, as has cardiac involvement [117, 118]. Diagnosis and management of liver disease are described before (Section Management) (Sect. 5 [115] and Sect. 6 [117–130].

Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Across all age groups, the most common hepatorenal fibrocystic disease is ADPKD, with an incidence of 1:1000. Liver involvement usually presents as polycystic liver disease, while CHF and CD or CS are rarely described. As its name suggests, transmission is dominant. The most common mutations are PKHD1 and polycystic kidney and hepatic disease 2 (PKHD2). All other mutations are related to the function of polycystin-1 [122, 123]. Kidney cysts develop progressively from the whole tubule. Onset is slow such that diagnosis is usually made in adulthood, with young women being the most severely affected [124, 125]. Other organs may also develop cysts: pancreas, intestinal epithelium, arachnoid mater, and seminal vesicles. Cardiac valvular disease and cerebral aneurysms have also been described. Affected patients are usually asymptomatic in childhood. On occasion, an incidental finding of cysts in the fetus, young child, or adolescent can lead to the misleading suspicion of ARPKD. Most often, in childhood, the incidental finding of a cyst should orient toward ADPKD, especially if hypertension is present. Some very rare cases of end-stage renal disease are described in childhood [131–134].

Nephronophthisis

Nephronophthisis is the most frequent genetic cause of end-stage kidney disease in children and may be associated with liver disease. Nephronophthisis is classified according to age at presentation: infantile, juvenile, and adolescent. In all cases renal involvement leads to end-stage renal disease requiring kidney transplantation. Most commonly, it is the adolescent form caused by mutations in nephrocystin 3 gene (NPHP3) which presents with liver disease characterized by bile duct proliferation and portal fibrosis [134–137]. This characteristic histology should encourage clinicians to search for NPHP3 mutations and examine renal function. Tapetoretinal abnormalities constitute another sign favoring the diagnosis [138]. Mutations in eight other genes have been identified in the adolescent and juvenile form nephrocystin 1,3–8 (NPHP1, NPHP3–NPHP8) but have not been associated with liver abnormalities except for an occasional biochemical abnormality. In NPHP2 and NPHP3, both presenting in infancy, liver involvement varies from mild to CHF [139–142].

Meckel Gruber Syndrome

Meckel syndrome is a rare autosomal recessive lethal ciliopathy composed of four cardinal features: occipital encephalocele, bilateral cystic kidneys, CHF (100%), and polydactyly. The incidence is 1:140,000 and is increased in the Finnish population (1:1000) [143–146].

Joubert and Coach Syndromes

Joubert syndrome (JS) is a recessive disorder with occurrence of 1:100,000 and characterized by brainstem and cerebellar malformation, ataxia, developmental impairment, alternance of hyperpnea and hypopnea, abnormal eye and tongue movements, and hypotonia and by an array of neurological, retinal, renal, or skeletal anomalies. The key feature of JS is molar tooth sign (cerebellar peduncles and deep interpeduncular fossa on cerebral MRI). The most frequent liver abnormality associated with JS is CHF and elevated serum aminotransferase levels. Cholangitis is very rare. Mutation in the transmembrane 67 (TMEM67) gene is strongly associated with liver involvement.

COACH syndrome includes *c*erebellar vermis hypo/aplasia, *o*ligophrenia, *a*taxia, *c*oloboma, and *h*epatic fibrosis. This very rare syndrome combines the presence of CHF and the classical neurologic signs of JS. Mutations in transmembrane protein meckelin (MKS3), a centriole protein necessary for ciliary function and for primary cilium formation, are present in more than 50% of COACH syndrome cases and are uncommon in JS patient without liver involvement [147–152].

Practical Approach

All patients with known cystic renal disease should be evaluated for liver involvement. Liver fibrosis begins early in life in patients with ARPKD and ADPKD. In the absence of signs or symptoms, it is not recommended to perform a liver biopsy. Portal hypertension should be sought at each visit. Annual liver function tests and annual liver ultrasonography are recommended. An MR cholangiogram should be performed once to understand biliary morphology, even when there are no significant findings on US. Although liver biopsy is the gold standard for diagnosis, it is not always indicated if it does not immediately alter management. Conversely, since the association between renal and liver diseases is so frequent, all patients diagnosed with CHF or CD/CS should be evaluated for renal cystic disease. Screening for malignant transformation is not indicated in children, because onset occurs generally in the fifth decade. Improved management of children with chronic liver disease means that more

patients with fibrocystic liver diseases are reaching adulthood with their native livers and therefore that adult gastroenterologists will be the ones to diagnose malignancy in these patients [153].

Genetics

Genetic testing is recommended for diagnosis, management, and prognosis [154]. If the clinical picture is suggestive of a given phenotype and the differential diagnosis narrow, known candidate genes can be tested to provide a definitive diagnosis, and genetic counseling should be offered to patients and relatives. Some associations between gene mutation and ciliopathies are strong as described in Table 60.3. But it can happen that the mutated gene does not correspond to the described phenotype of the ciliopathy. Indeed, there is phenotypic overlap across genotypes, something highlighted in Table 60.3. When the differential diagnosis is broader, casting a wider net to identify the ciliopathy is easily achievable through next-generation sequencing (NGS). Given the complexity of these analyses and the inherent difficulty of interpretation, clinical genetic expertise is advisable. According to the identified variant and the specific mode of inheritance, familial segregation analysis may be indicated.

Conclusion and Future Perspectives

Ciliopathies often present with liver disease due to associated ductal plate malformation. Associated renal disease is frequent and should be sought. Liver involvement must be recognized as it may progress to life-threatening complications (portal hypertension or cholangitis) and possibly warrant liver transplantation. An increasing number of disease causing mutations are identified, and sequencing is part of the diagnostic workup of these diseases. However, there is no clear genotype-phenotype correlation. In particular, it is unclear why some ciliopathies present with distal DPM, while others show abnormalities of the large, proximal bile ducts.

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Familial Intrahepatic Cholestasis

61

Tassos Grammatikopoulos

Abbreviations

BSEP	Bile salt export pump
CLDN1	Claudin-1
CLDN2	Claudin-2
FHC	Familial hypercholanaemia
FIC	Familial intrahepatic cholestasis syndrome
FXR	Farnesoid X-activated receptor
GGT	Gamma-glutamyltransferase
HCC	Hepatocellular carcinoma
IE	Ileal exclusion
LT	Liver transplantation
MDR3	Multidrug resistance protein 3
MYO5B	Myosin 5B
PEBD	Partial external biliary diversion
SPAD	Single pass albumin dialysis
TJP2	Tight junction protein 2
UDCA	Ursodeoxycholic acid
USP53	Ubiquitin-specific peptidase 53
ZO	Zona occludens

Introduction

Two main mechanisms of cholestasis have been so far identified, which can be either hepatocellular or obstructive in origin affecting the biliary structures. Hepatocellular cholestasis is considered to be due to alterations in bile formation and transportation of bile salts and other components of bile at the cellular level involving multiple mechanisms. Obstructive cholestasis on the other hand is due to bile duct injury either at extra- and/or intrahepatic level.

To date, seven major types of familial intrahepatic cholestasis have been identified, associated with mutations in genes encoding proteins involved in the hepatocellular transport and structure. These are FIC1 deficiency, bile salt export pump (BSEP) deficiency, multidrug resistance protein 3 (MDR3) deficiency, tight junction protein 2 (TJP2) deficiency, farnesoid X-activated receptor (FXR) disease, myosin 5B (MYO5B) disease and ubiquitin-specific protease 53 (USP53)-associated liver disease. The inheritance pattern in each case is autosomal recessive. Presentation is usually within the first year of life for the more severe forms either in a recurrent, such as FIC1 and MYO5B, or progressive form such as BSEP, TJP2 and MDR3 deficiency. Irregularities in bile formation and transportation can lead to disturbance in the enterohepatic circulation with bile salt retention and cholestasis. Cholestatic syndromes can affect solely the liver but also can have multi-organ involvement (Table 61.1).

FIC1 Deficiency

Familial intrahepatic cholestasis protein 1 (FIC1) deficiency is an autosomal recessive condition with a wide phenotypic spectrum extending from mild recurrent to severe liver disease. FIC1 deficiency was initially called Byler's disease, after the Amish family in which it was first described. Furthermore, the term Byler's bile has been used as the description of coarsely granular canalicular bile seen in FIC1 deficiency on transmission electron microscopy.

It is characterised by hepatocellular cholestasis with low serum levels of γ-glutamyltransferase (GGT) activity and normal cholesterol levels. Presentation can be early on in life with recurrent cholestatic episodes and eventually progress to cirrhosis [1, 2]. Less severe disease can however present in adulthood. Presenting features consist of cholestasis, low serum albumin, epistaxis and splenomegaly.

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Table 61.1 Seven major types of familial cholestatic syndromes with respective organ involvement and disease severity

Disease name	Aliases	Gene	Protein	Features
FIC1 deficiency	Byler's disease, PFIC1	*ATP8B1*	FIC1	Multisystem disorder Wide range of severity
BSEP deficiency	Byler's syndrome, PFIC2	*ABCB11*	BSEP	Liver only affected Spectrum of severity
MDR3 deficiency	PFIC3	*ABCB4*	MDR3	Liver only affected Wide range of severity
TJP2 deficiency	PFIC4	*TJP2*	TJP2	Multisystem disorder Wide range of severity
FXR liver disease	Farnesoid X-activated receptor associated liver disease PFIC5	*NR1H4*	FXR	Rapid-onset progressive liver disease
MYO5B liver disease	PFIC6	*MYO5B*	MYO5B	GI and liver disease Variable range of severity
USP53-associated liver disease	PFIC7	*USP53*	USP53	Multisystem disorder Mild disease severity

FIC1 is the protein encoded by the gene *ATP8B1* [3–5], which is located at chromosome 18q21-q22. FIC1 (or ATP8B1) is a P-type ATPase, which is one of several aminophospholipid flippases present in different membranes. Its role in the liver, largely based on a mouse model, is in maintaining the gradient of phosphatidylethanolamine and phosphatidylserine towards the inner leaflet of the cell membrane. A transmembrane balance of lipids appears to be required in the presence of the high bile salt concentration in the canaliculus [6]. The normal canalicular membrane requires a high degree of detergent resistance in order to prevent damage from the detergent effect of bile itself. Paulusma et al. showed that by ex vivo infusion of bile acids in the knockout mouse, the extraction of aminophospholipids was greatly increased. In the human liver, several proteins are not present, or are in reduced quantities, in the canalicular membrane of patients with FIC1 deficiency. This observation still does not account for the cholestasis, as no transporter critical to bile formation has been identified to be absent. It appears more likely that these proteins are malfunctioning in an altered lipid environment of the canalicular membrane. As FIC1 deficiency is primarily characterised by cholestasis, studies have attributed this phenotype to a defective farnesoid X-activated receptor (FXR) signalling pathway [7, 8]. Other groups however suggested that impaired FXR activity is secondary to cholestasis and not responsible for the phenotype [9]. The activity of FXR and its target genes remained uninterrupted in FIC1-depleted Caco-2 cells, created using small hairpin RNA and small interfering RNA, respectively, suggestive of an unimpaired FXR signalling mechanism in FIC1-deficient patients [10, 11]. In view of a proportion of phenotypically FIC1-like patients in whom no mutations were identified, a study looked into promoter and 5′ untranslated (5′-UTR) regions affecting gene regulation in human liver and small intestine tissue by 5′ rapid amplification of cDNA ends. Expression levels of *ATP8B1* transcripts were determined by quantitative reverse-transcription PCR and compared with the non-variable part of *ATP8B1*. Twelve different splicing variants and four novel untranslated exons located up to 71 kb upstream of the previously published exon 1 were identified in both tissue types. A number of transcription start sites were identified, and the proximal promoter upstream of the major transcription start sites was also proven to be an essential regulatory element responsible for 70% of total *ATP8B1* transcriptional activity. In vitro the main promoter was shown to drive constitutive *ATP8B1* gene expression independent of bile acids [12].

Altered gene expression demonstrated in extrahepatic sites may be contributory to the multisystemic nature of this cholestatic syndrome. Other disease features include diarrhoea, malabsorption of fat-soluble vitamins, hearing loss, pancreatitis, renal tubular acidosis, delayed puberty and growth failure. The multisystem nature of the disease is supported by the widespread expression of the *ATP8B1* gene including the liver, pancreas, kidney and more widely in the small intestine [5].

Liver histology changes consist of cholestasis with bile plugs and periportal biliary metaplasia of hepatocytes in the absence of ductular proliferation in the portal tracks (Figs. 61.1, 61.2, and 61.3).

Early-onset patients, without treatment, usually progress to end-stage liver disease by early adulthood. The first-line treatment includes antipruritic agents such as ursodeoxycholic acid (UDCA) [13], fat-soluble vitamin supplementation and nutritional support. Failure of medical treatment would lead to the first-line surgical treatment, which is currently partial external biliary diversion (PEBD), a technique that diverts an unknown proportion of bile externally. The consequences of this procedure certainly include interruption of the enterohepatic circulation of bile acids [14–17]. An external stoma can also be formed by using the appendix creating a cholecystoappendicectomy as reported by Rebhandl et al. [18]. Another alternative is ileal exclusion (IE), which has also been performed in cases with previous cholecystectomy or as rescue treatment [2, 15, 19, 20].

Liver transplantation (LT) is the next treatment option for these patients [16], but some extrahepatic symptoms will not

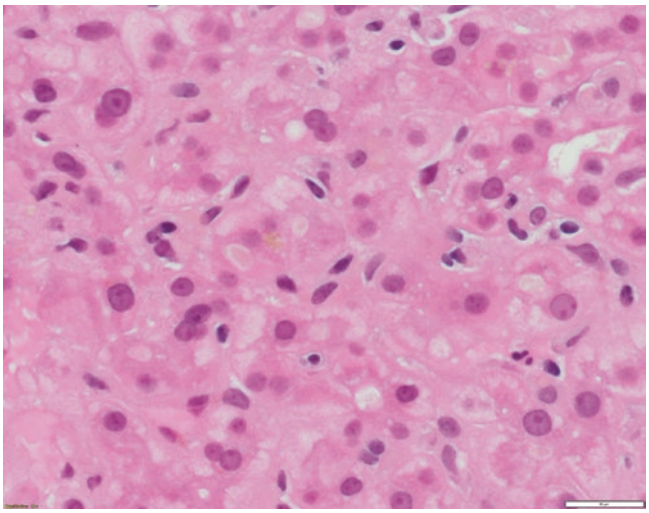

Fig. 61.1 Liver biopsy from a 20-year-old patient with ATP8B1 disease/FIC1. Hepatocytes are small and without anisocytosis. There is canalicular cholestasis. Of note, there is pale staining bile within the canaliculi. [H&E ×400 magnification]

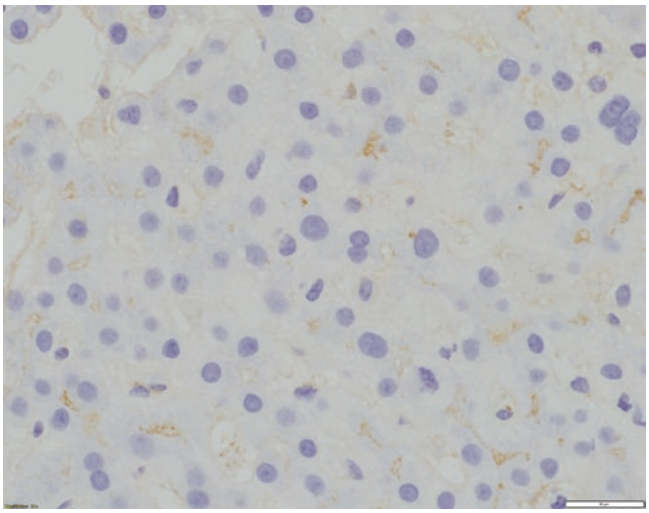

Fig. 61.2 Absence of canalicular GGT expression. ×400 magnification

improve, such as growth, and some may even become aggravated, such as diarrhoea [14], liver steatosis or kidney disease. Some of these patients may end up requiring multi-organ transplantation including liver, small bowel, kidney and pancreas (personal data).

BSEP Deficiency

The major bile salt transporter of the canalicular membrane level is the BSEP, which is encoded by the gene *ABCB11* [21]. The clinical condition associated with bile salt transport deficiency was previously known as "Byler's syndrome". The phenotype of BSEP-deficient patients can also vary from a mild

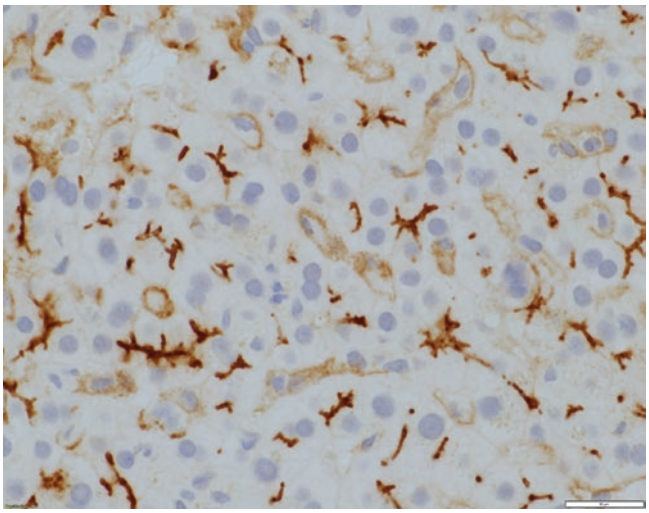

Fig. 61.3 Evident canalicular expression of CD10 [×400 magnification]

(BRIC) to a more severe type requiring liver transplantation [22–24]. *ABCB11*, expressed only in the liver, encodes BSEP, a member of the ATP-binding cassette (ABC) family of transporters responsible for the transport of bile acids across the canalicular membrane. The locus for *ABCB11* is at chromosome 2q24 with the most common European mutations being D482G (c.1445A > G) and E297G (c.890A > G) [21, 23]. BSEP deficiency leads to accumulation of bile salts within hepatocytes and has a subsequent effect on hepatocellular function. As anticipated, it has also been shown to have significant affinity for some of the main bile salts in human bile, such as glycocholate, taurocholate and chenodeoxycholate [25].

Severe BSEP deficiency presents within the first year of life, and, although variable, it is usually a non-relapsing severe progressive cholestasis and pruritus leading to fibrosis. Birth weight can be below normal range especially in the non-D482G > E patients [22]. Serum bilirubin levels are not necessarily reflective of the degree of cholestasis as bilirubin is transported separately from bile salts. Like FIC1 deficiency, BSEP-deficient patients demonstrate low γ-glutamyltransferase (GGT) activity but with a trend to higher cholesterol, significantly raised transaminases (>threefold higher compared to FIC1 deficiency), alpha-fetoprotein and serum bile acid concentrations. Other biochemical indices include fat-soluble vitamin deficiencies manifesting with coagulopathy and rickets. Gallstones have been reported in up to 32% of patients [22]. Serum bile acid profiles demonstrate a high cholic acid-to-chenodeoxycholic acid ratio as reported in FIC1 patients. Reduced biliary bile salt concentration in BSEP patients is similar to that in PFIC1 and in direct contrast to MDR3 deficiency patients [3, 26, 27].

Severe phenotypes have been associated with mutations leading to protein truncation or failure of protein production.

In a series by Strautnieks et al., missense mutations were identified in 79% of patients, many affecting protein processing and trafficking or protein structure [28]. Previous reports of *ABCB11* missense mutations and single nucleotide polymorphisms showed pre-mRNA splicing subsequently causing reduction in mRNA levels in a significant number of cases. These defects at the protein or mRNA level can have a detrimental impact in BSEP function [28].

Liver histology features consist of increased lobular inflammation and portal fibrosis, giant cell transformation of hepatocytes and neonatal hepatitis with finely filamentous bile. The absence of liver immunohistochemistry for BSEP can assist in the diagnostic process [23, 29] (Fig. 61.4). Immunohistochemically detectable BSEP expression does not exclude functional BSEP deficiency [30]. In a previous series, 28% of BSEP patients analysed exhibited some degree of BSEP staining, and in a small minority expression was even considered normal [23].

Hepatocellular carcinoma (HCC) or cholangiocarcinoma has been reported in a number of children on the background of severe neonatal hepatitis [31, 32] or proven BSEP deficiency [23, 32]. The risk appears to be higher in patients with two protein-truncating mutations (38% vs. 10%) compared to other genotypes. The exact mechanism of malignancy remains unclear, though further insight to the development of these tumours has been gained recently [33].

Treatment options for BSEP deficiency include antipruritic agents such as UDCA, rifampicin, liver enzyme inducers (phenobarbitone), nutritional support and fat-soluble vitamin supplementation, where indicated. Partial external biliary diversion, ileal exclusion and liver transplantation are all recommended surgical treatment options [16, 20, 34, 35]. Hepatocellular malignancy remains an indication for early liver transplantation in BSEP patients. Patients with biallelic truncating mutations warrant close monitoring with liver ultrasonography and serum alpha-fetoprotein levels. Overall, in terms of prognosis, patients with D482G mutation developed portal hypertension less frequently, developed fibrosis at an older age and underwent liver transplantation at an older age as well [22].

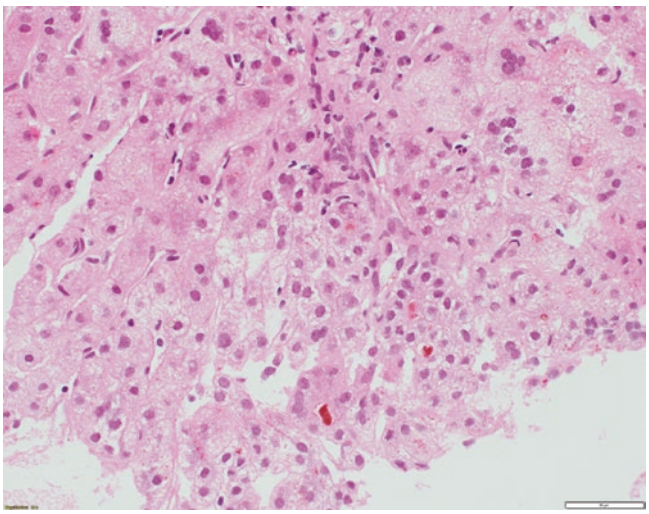

Fig. 61.4 Liver biopsy from a 7-month-old patient with ABCB11 disease/BSEP deficiency. There is bridging fibrosis and partial nodular transformation of the parenchyma (not shown). Hepatocytes show giant cell change and canalicular cholestasis. Lobular inflammation is present. BSEP immunostaining shows the absence of canalicular expression, whilst canalicular GGT expression is preserved (not shown) [H&E stained slide at ×200 magnification]

Recurrence of symptoms after LT has been described in BSEP-deficient patients, in contrast to other FIC syndromes. Jara et al. reported recurrence of cholestasis and pruritus following LT in BSEP patients with no evidence of cellular rejection on liver biopsy in 2009 [36]. In the same year, BSEP antibodies in the serum and at the hepatocyte canalicular membrane of a single patient who underwent two liver transplants were described [37]. The mechanism of action is thought to be that anti-BSEP antibodies form, which bind to an epitope in the intracanalicular domain of BSEP and subsequently block the function of the bile acid transporter. A multicentre series of six patients with recurrence of disease [38] was reported in 2010. In all of these patients, treatment was extremely problematic, four underwent a repeat LT and various management protocols were suggested. All reports have identified mutations (splice site, missense, truncating) leading to the absence of BSEP protein expression on immunostaining. Modifications in immunosuppression, plasmapheresis, intravenous immunoglobulin courses and single pass albumin dialysis were used in isolation or jointly with limited effect on patients' symptoms. Following the identification of BSEP antibodies in post-LT BSEP deficiency patients with cholestasis in the absence of rejection, an antibody-based treatment was suggested as potentially beneficial. Two cases of patients with BSEP deficiency that, following LT, had demonstrated evidence of functional BSEP deficiency treated successfully with two repeated 4-week courses of anti-CD20 monoclonal antibodies were subsequently reported [39, 40].

TJP2 Deficiency

Since in one third of patients with normal-GGT cholestasis mutations in either *ABCB11* or *ATP8B1* have not been identified [26], a cohort of 33 cholestatic children with relatively low serum GGT levels were studied recently by Sambrotta et al. [41]. From eight consanguineous families with novel protein-truncating mutations in the tight junction protein-2 gene (*TJP2*), located in chromosome 9q21.11, 12 patients were identified. The phenotype of these patients consists of early presentation within the first couple of months of life with low serum GGT, and nine of

them underwent liver transplantation by their first decade of life. Patients can develop portal hypertension, persistent pruritus and malabsorption. A single patient died at 13 months. Liver immunohistochemical findings consist of lack of TJP2 and reduced staining of claudin-1 (CLDN1) with normal distribution of claudin-2 (CLDN2), both proteins essential to cellular tight junctions (Figs. 61.5, 61.6, and 61.7). On transmission electron microscopy, elongation of the tight junctions with sparsity of the zona occludens (ZO) is also seen. Extrahepatic involvement is present in some cases and consists of chronic respiratory disease or neurological complications such as subdural haematomas. Mutations in the *TJP2* gene have been previously associated with familial hypercholanaemia (FHC), a nonprogressive cholestatic disorder, described in the Amish population [42]. In that report, out of all 17 individuals with FHC screened, 11 patients in eight families were found homozygous for an incompletely penetrant missense mutation in *TJP2,* with alterations in the cellular bile acid concentration gradient. Mutations in the *TJP2* gene are also known to cause intrahepatic cholestasis of pregnancy (ICP) [43], BRIC [44], cirrhosis in adults and hepatocellular carcinoma [43, 45, 46] and deafness [47, 48].

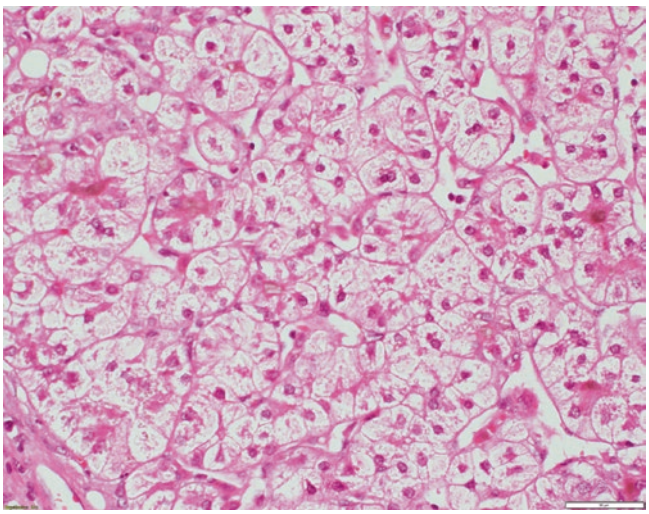

Fig. 61.5 Liver biopsy from a 4-year-old patient with TJP2 disease. There is extensive bridging fibrosis (not shown). Hepatocytes are oedematous and show rosetting. Canalicular cholestasis is present. [H&E ×200 magnification]

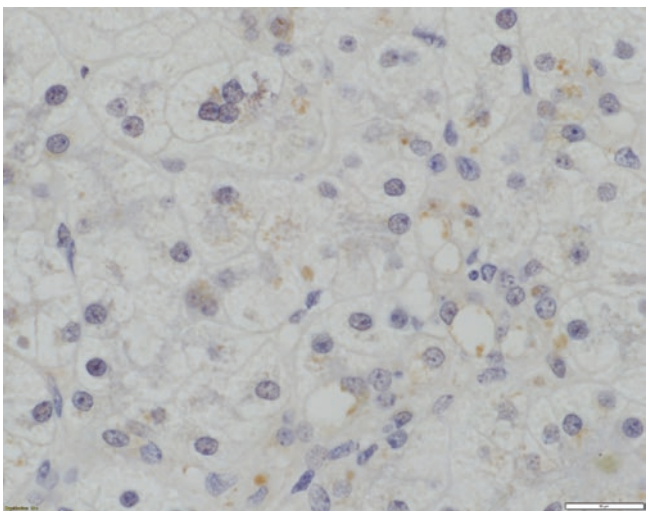

Fig. 61.6 Absence of canalicular TJP2 expression. [×400 magnification]

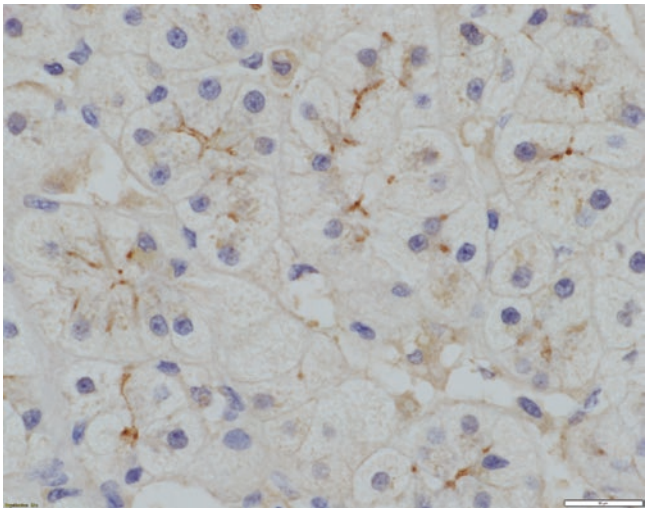

Fig. 61.7 With expression of canalicular BSEP [×400 magnification]

Treatment consists of supportive choleretic agents, fat-soluble vitamin supplementation, nutritional support, partial external biliary diversion (PEBD) and subsequently liver transplantation. No liver malignancy has been so far described in this newly defined PFIC group.

MDR3 Deficiency

This type of FIC is caused by a variety of mutations in the ATP-binding cassette subfamily B member 4 (*ABCB4*), the gene encoding multidrug resistance protein 3 (MDR3) [49]. It is also inherited in an autosomal recessive pattern. *ABCB4* has been mapped to the 7q21-36 region, and it codes for a floppase responsible for phosphatidylcholine [50] translocation across the canalicular membrane. Defective PC translocation leads to a lack of PC in bile. The absence of PC inhibits the chaperoning of bile acids through micelle formation, leading to damage to the biliary epithelium and cholangiopathy. Biliary phospholipid levels are significantly reduced, and biliary bile salt-to-phospholipid and cholesterol-to-phospholipid ratios are significantly higher in affected individuals when compared with wild-type bile [51].

MDR3 deficiency causes a spectrum of liver diseases such as cholesterol cholelithiasis, adult biliary cirrhosis, low-phospholipid-associated cholelithiasis syndrome (LPAC), transient neonatal cholestasis, intrahepatic cholestasis of pregnancy (ICP) and drug-induced cholestasis [52–58]. In severe MDR3 deficiency, symptoms can manifest within the first year, but not usually as neonatal jaundice, and gradually progress towards liver cirrhosis and end-stage liver disease within the first few years of life [1, 59, 60]. Patients with a single affected copy of the gene can develop symptoms under particular circumstances, such as pregnancy, whilst otherwise they may remain asymptomatic [61, 62].

Liver histology demonstrates expansion of portal tracts, bile duct proliferation, bile plugs and portal fibrosis with mixed inflammatory infiltrate (Fig. 61.8). Cytokeratin immunostaining can be confirmatory of the ductular proliferation, and MDR3 immunohistochemistry can be absent or markedly reduced at the canalicular membrane in affected individuals [63].

MDR3 deficiency is differentiated from the other three FIC types at a biochemical level by an elevated serum of γ-glutamyltransferase (GGT). Biochemical profile also consists of normal serum cholesterol and moderately raised concentration of serum primary bile salts [27].

The first-line management, as in the other FIC types, is with UDCA and other antipruritic and choleretic agents. UDCA seems to be very effective in milder cases, and it may also prevent disease progression in these cases. Liver transplantation remains the treatment of choice for the nonresponders.

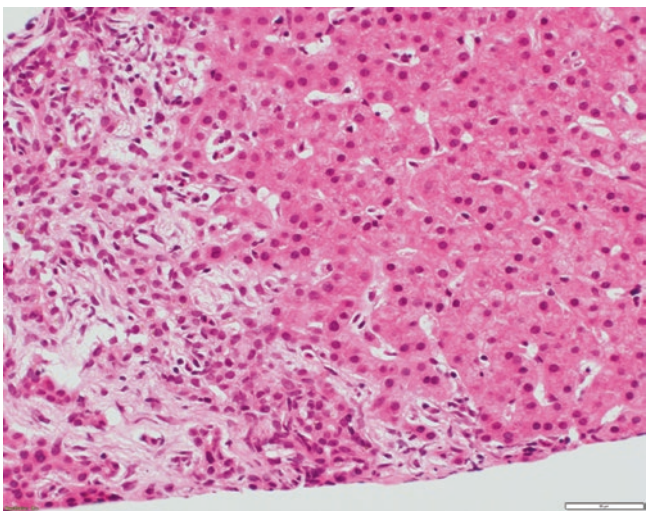

Fig. 61.8 Liver biopsy from a 1-year-old child with MDR3 deficiency/ABCB4 disease. There is a cholangiopathy manifested by cholangiocyte disarray and vacuolation. Immunohistochemistry demonstrated an absence of canalicular MDR3 expression (not shown) [H&E ×200 magnification]

Farnesoid X-activated receptor

Another molecule regulating bile acid homeostasis is the farnesoid X-activated receptor (FXR). FXR belongs to a subclass of metabolic receptors within the intracellular ligand-activated nuclear receptor (NR). FXR is encoded by the nuclear receptor subfamily 1, group H, member 4 *(NR1H4)* gene, located in chromosome 12q23.1 [64, 65]. Bile acids are ligands for the nuclear hormone receptor FXR, which together with its heterodimeric partner, the RXR, acts as a transcription factor for several bile salt transporters, including the hepatic BSEP and the ileal bile acid binding protein (I-BABP) [66, 67]. The expression of short heterodimeric protein (SHP)-1 in the liver, which acts as a transcriptional repressor, is itself regulated by FXR and can downregulate the expression of several genes including Ntcp and cholesterol-7a-hydroxylase CYP7A1, which is a rate-limiting enzyme in bile salt synthesis [68]. Conversion of cholesterol to bile salts by CYP7A1 is stimulated by the oxysterol-activated liver X receptor (LXR). Bile salts have a negative effect on their synthesis by activation of the FXR-dependent short heterodimeric protein 1 (SHP-1), which in turn suppresses *CYP7A1* and 8B1 transcription. The activation of FXR by bile salt production stimulates the transcription of Mrp2, Bsep and OATP8 [69, 70]. Bile salt synthesis is also regulated by a feedback mechanism between FXR and other nuclear receptors including RXR, LXR and LRH-1.

FXR is expressed in the liver, intestine and kidney, and its activation has been shown to be activated by both conjugated and free bile salts such as chenodeoxycholate, deoxycholate, lithocholate and cholate [67]. There are similarities in the activation of FXR by bile salts in hepatocytes and terminal ileum enterocytes which in essence controls the entire enterohepatic circulation of bile salts [71]. The presence of bile salts in the terminal ileum activates FXR which reduces bile salt uptake and promotes their secretion via the basolateral membrane reducing the intracellular concentration and increasing their excretion via the faecal route [72, 73]. FXR has been shown to activate an endocrine feedback mechanism via the fibroblast growth factor 19 (FGF19). FGF19 is secreted from the ileum to the portal venous system whereby once it reaches the liver it supresses bile salt production by activation of the FGF receptor 4 [74]. FGF19 can also activate hepatic glycogen synthesis and inhibits gluconeogenesis [75, 76].

Gomez-Ospina et al. reported the first small series of four individual patients from two unrelated families with homozygous loss-of-function variants in *NR1H4* [65]. All patients presented at a very early age. Three presented with neonatal cholestasis and one presented with ascites, pleural effusions and intraventricular haemorrhage at birth. At the time of initial evaluation, all patients had conjugated hyperbilirubinemia, raised liver transaminases, low-to-normal GGT activity

and refractory to vitamin K coagulopathy. Two patients underwent successfully LT, one died on the waiting list for LT and another patient died at 5 weeks of age from an acute vascular event. Chen et al. identified one patient with a single heterozygous nonsense variant in *NR1H4* from a large cohort of children with early-onset normal GGT activity cholestasis [64]. Their patient underwent biliary surgery and T-tube drainage for refractory to medical treatment cholestasis with minimal effect. Subsequently, this case developed cirrhosis and ascites. So far, the phenotype of patients affected with FXR-associated liver disease seems of early-onset cholestasis leading to cirrhosis and LT.

Liver histology can show ductular reaction, giant cell transformation with hepatocyte ballooning and intralobular cholestasis. Biopsy material from explanted livers had features of micronodular cirrhosis and fibrosis. Liver immunohistochemistry showed lack of canalicular BSEP expression with preservation of MDR3.

More recently, FXR agonists, such as the bile salt derivative obeticholic acid, have been shown to improve alkaline phosphatase (ALP), a disease marker in primary biliary cirrhosis [77, 78], but with minimal effect on pruritus. In recent years, there have been studies suggestive of FXR's potential therapeutic role also in lipid and glucose metabolism [79, 80], immunomodulation in inflammatory bowel disease [81, 82] and colorectal cancer [83].

Further reports are required to expand our knowledge of the disease, understand genotype-phenotype associations and investigate potential therapeutic options.

Myosin 5B Cholestasis

MYO5B is a motor protein-mediating membrane transport and recycling in polarised cells, found in all epithelia [84] and encoded by the gene *MYO5B,* located in 18q21.1. MYO5B binds selected small guanosine triphosphatase (GTPase) rab proteins, including the trans-Golgi network (TGN)-associated and the recycling endosome-associated rab11a and rab8, and has been implicated in protein trafficking across the apical plasma membrane [85].

Functional deficiency or loss of MYO5B results in aberrant cell polarity and is the major cause of microvillus inclusion disease (MVID), an autosomal recessive disorder causing watery diarrhoea presenting in early infancy that requires parenteral nutrition. The condition can also lead to intestinal failure and small bowel transplantation [86, 87]. Loss of the motor function causes the formation of inclusions and retention in brush border enzymes that lead to early-onset watery diarrhoea and failure to thrive [88].

Cholestasis when reported in patients with MVID is often considered identical to the typical intestinal failure-associated liver disease phenotype. More recently though, there has been a clearer distinction in this type of cholestatic syndrome, now called MVID-associated cholestasis [89, 90]. Recent evidence shows that the effects of mutations on *MYO5B* (OMIM 606540) are not limited to the small intestine but can also involve other organs such as the stomach, colon, pancreas and more importantly the liver [84]. Interestingly, a small group of children can present with MVID-like disease, but although they recover from their gastrointestinal disease with histologically normal small intestine, they suffer from recurrent cholestasis and troublesome pruritus [91].

Patients can present with cholestasis and pruritus at an early age but some at a much later stage. The biochemical profile is that of low GGT activity with preserved synthetic function and evidence of cholestasis with raised bilirubin or serum bile acids and limited parenchymal inflammation (AST/ALT) and gallstones. The difference in the disease phenotype between MVID- and MYO5B-associated cholestasis has not been yet clearly defined though, and further functional studies are required.

It has been recognised that deficiency of MYO5B impairs the BSEP in the canalicular membrane contributing to cholestasis [92]. BSEP is a liver-specific transporter and crucial to bile acid transport at the canalicular membrane [93]. An alternative mechanism for the cholestasis in these patients has also been attributed to impairment of MYO5B/RAB11A interaction [92, 94, 95]. The degree of jaundice or cholestasis can be variable amongst patients with MYO5B-associated cholestasis but somewhat comparable to other low-GGT cholestatic syndromes such as FIC1 and BSEP deficiency [5, 96]. There are often clinical similarities between their MYO5B cohort and patients with other low-/normal-GGT cholestatic syndromes but also differences in their histological/immunostaining findings. Liver histology findings are those of mild and nonspecific portal inflammation with minimal, focal lobular cholestasis and fibrosis. BSEP expression has been variable amongst different case series from preserved and localised to the canalicular membrane to distortion with low GGT expression mainly identified in periportal areas [94].

Croft et al. reported a unique case, where the patient presented with severe gastrointestinal symptoms, typical of MVID, which resolved over childhood. The GI histology showed MYO5B expression at the microvillus brush border, and ultrastructural studies confirmed the absence of microvillus inclusions, with intact normal-sized microvilli in the brush border [91, 96]. This case subsequently developed cholestasis and pruritus. Perry et al. reported some atypical cases of MVID in whom one patient had transient intestinal failure but was on full enteral feeds at time of reporting [97]. Both these reports highlight the clinical diversity of GI involvement in patients with MYO5B cholestasis in whom a convincing pathophysiologic explanation remains to be established.

The clinical progression of the disease demonstrates a variable response to medicinal antipruritic agents and surgical interventions. Some centres reported good response to medicinal agents (bile acid modifiers); others explored the potential role of surgical interruption of the enterohepatic circulation (external or internal biliary diversion) and nasobiliary drainage with limited effect on pruritus and quality of life and a minority progress to LT [98–100]. The clinical progression of the disease demonstrates a variable response to medicinal antipruritic agents (UDCA, cholestyramine, rifampicin, naltrexone, chlorphenamine) and surgical interventions. Some patients require biliary diversion with limited effect on pruritus and quality of life, but only two reported cases so far progressed to LT. Despite the various treatment modalities, patients do not become completely asymptomatic. Clinical trials currently underway in other cholestatic conditions interfering with the bile acid homeostasis may have a beneficial effect on children with *MYO5B*-associated cholestasis.

The long-term prognosis of patients with *MYO5B*-associated liver disease should be addressed with caution on the basis of the uncertainty whether these patients will remain free of GI symptoms in the future and their liver disease outcome in adulthood.

Ubiquitin-Specific Peptidase 53-Associated Liver Disease

In the last 2 years, another type of progressive familial intrahepatic cholestasis with normal GGT has been described in association with variants in the ubiquitin-specific peptidase 53 gene [101–104]. To date, there have been three case series reported describing a limited number of patients with *USP53*-related cholestasis.

The *USP53* gene, located in chromosome 4q26, encodes a non-protease protein homologue of the ubiquitin-specific peptidase family [105] important for the regulation of protein degradation, but due to lack of a histidine residue, this function is lost [106]. The USP53 protein is expressed in the kidney, inner ear, liver and brain. USP53, a tight junction (TJ) protein, co-localises and interacts at the cellular TJs with other tight junction proteins 1 and 2 (TJP1 and TJP2). In a mouse model, Usp53 is interacting with TJs and more specifically with TJP2. The missense variant c.682T > A; p.Cys288Ser seems though to not alter the co-localisation with TJP2, and it has been reported to cause progressive hearing loss [107].

The biallelic variants described so far include mostly homozygous or compound heterozygous forms with variations though amongst reports. Bull et al. and Alhebbi et al. reported in all but one homozygous changes in *USP53* variants causing loss of function unlike the series by Zhang et al. where all cases were compound heterozygous in variants causing either nonsense, frameshifting, canonical splicing or missense changes. In view of the limited cases reported, it remains challenging to extract meaningful phenotype-genotype associations and more so about the long-term prognosis.

Clinical presentation can include relapsing normal-GGT cholestasis varying from early infancy to adolescence, pruritus and signs of fat-soluble vitamin deficiency such as hypocalcaemia, tetany and intracranial bleeding due to vitamin D and K deficiency, respectively. Hypertrophic cardiomyopathy, heart failure, hypothyroidism, unilateral kidney enlargement, developmental/speech delay and hearing loss requiring cochlear implants have also been reported. The biochemical profile of patients with *USP53*-associated liver disease consists of high serum bilirubin and ALP, mildly elevated ALT/AST and normal GGT [101–104].

Liver histopathology shows hepatocyte giant cell changes, canalicular and hepatocellular cholestasis with mild lobular activity and in a single case [101] cholangiopathy with florid duct proliferation and inflammation. Of interest, there is also porto-septal and porto-portal fibrosis and parenchymal nodularity hinting towards the chronic nature of the liver disease.

Immunohistochemistry confirms canalicular protein GGT and BSEP expression in a pan-lobular distribution in all patients. TJP2 can be expressed albeit decreased and less crisp with challenges in USP53 immunostaining from reports [102, 104]. In ultrastructural studies, Bull et al. reported variations in bile retention (granular or finely filamentous) with preservation of tight junctions, but changes in TJ's length and orientation were described by Zhang et al.

Patients reportedly respond well to UDCA and/or cholestyramine and rifampicin with relapse in pruritus after temporarily stopping treatment.

In the majority of patients with *USP53*-associated cholestasis, the disease phenotype may be relatively mild albeit relapsing, but one patient has already undergone LT. There is also variability in onset of hearing deficit, and further reports will enhance our knowledge and understating of this newly described condition.

Conclusion

Over the last few years, our understanding of paediatric cholestatic disorders has significantly improved. Through the latest gene sequencing techniques (targeted resequencing and/or whole exome sequencing) and careful patient selection, we have managed to identify causative genes for cholestatic syndromes in most patients and highlight the mechanism of action of the encoded proteins at a cellular level. This enables physicians to characterise and treat these patients more efficiently. A number of clinical trials are cur-

rently underway in some of the above-mentioned cholestatic conditions interfering with the bile acid homeostasis, and yet there is to be established the application of possible gene therapies. Whether such treatment options would be beneficial to children with FIC1, BSEP deficiency or other cholestatic syndromes requires further interrogation. Additionally, new clinical challenges are appearing such as recurrence of BSEP deficiency after liver transplantation.

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Alagille Syndrome

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Introduction

Alagille syndrome (ALGS) is an autosomal dominant phenotypically heterogeneous disorder. The defining clinical features of ALGS as described by Daniel Alagille include cholestatic liver disease classically with bile duct paucity, cardiac disease, posterior embryotoxon, butterfly vertebrae, and characteristic facial features [1]. The diagnostic criteria have since been expanded to encompass vascular and renal anomalies [2, 3]. ALGS stems from heterozygous mutations in one of two genes in the Notch signaling pathway: *JAGGED1* (*JAG1*) mutations are reported in up to 94% of clinically diagnosed cases, and mutations in the *NOTCH2* receptor are found in 2.5% [4]. *De novo* mutations account for approximately 60% of ALGS cases.

Historically, the prevalence of ALGS has been estimated to be approximately 1 in 70,000 live births. However, advances in molecular diagnostics have revealed that the true burden of ALGS is likely closer one in 30,000 [5]. This increase in prevalence is partially attributable to the growing availability and use of genetic testing and capturing individuals who present with partial or subclinical disease expression. There is remarkable variability in both disease severity and organ system involvement among ALGS patients, even within families harboring the same pathogenic variant. The following sections will describe the hallmark features of ALGS and provide evidence-based management considerations and strategies.

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Clinical Manifestations

Hepatic

The majority of ALGS patients who are symptomatic with liver disease present in the first year of life with cholestasis, classically with conjugated hyperbilirubinemia and high γ-glutamyltransferase (GGT). During childhood, elevations of serum bilirubin up to 30 times normal and serum bile salt elevations of 100 times normal are common. Cholesterol levels can be staggering and may exceed 1000–2000 mg/dL. However, this high level of plasma cholesterol is largely associated with lipoprotein-X [10]. Lipoprotein-X is in the low-density lipid range and resists oxidation, thereby protecting against atherosclerosis. Thus, the hypercholesterolemia of ALGS does not appear to carry an increased risk of cardiovascular disease [6, 11]. Hepatic synthetic function is usually well preserved in ALGS.

Physical examination findings in children with ALGS and liver disease include hepatomegaly early on and splenomegaly in the majority over time. The pruritus seen is among the most severe of any liver disease. It rarely is present before 3–5 months of age but is seen in most children by the second year of life, even in some who are anicteric. Multiple xanthomas are common sequelae of severe cholestasis associated with ALGS and correlate with a serum cholesterol level greater than 500 mg/dL. Xanthomas typically form on the extensor surfaces of the fingers, the palmar creases, the nape of the neck, the ears, the buttocks, and around the inguinal creases (Fig. 62.1a, b). Xanthomas are disfiguring and occasionally interfere with fine motor function when they occur on the fingers and feet.

Bile duct paucity is the histopathologic hallmark of ALGS and is defined as a ratio of interlobular bile ducts to portal tract ≤0.5 in at least five portal tracts (normal ratio 0.9–1.8). Other histological features of ALGS include giant cell transformation and, occasionally, biliary obstruction in patients with a biliary atresia (BA) phenotype. Notably, bile duct

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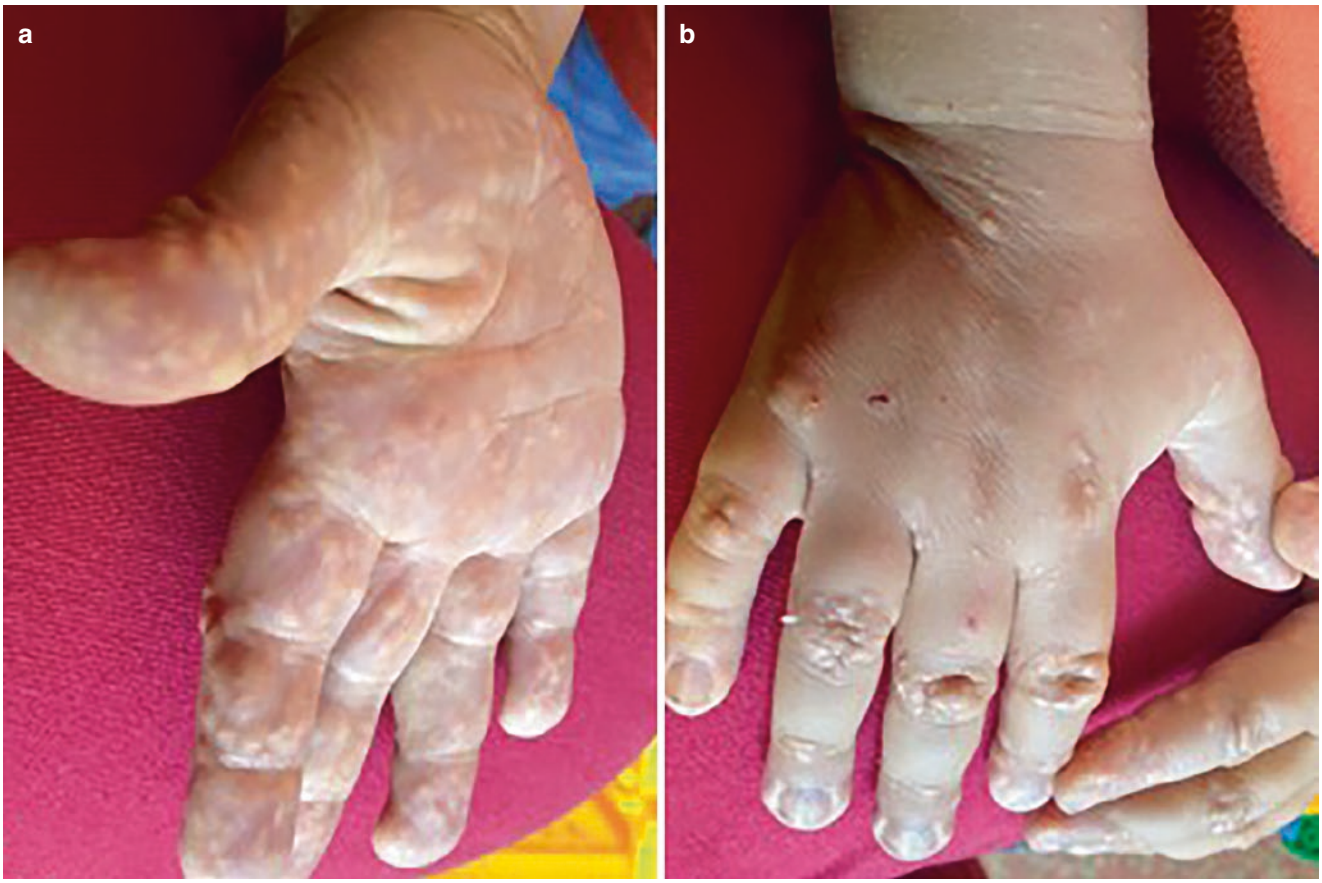

Fig. 62.1 (**a, b**) Xanthomas on the hands of a child with Alagille syndrome

paucity is not always present in early infancy and has been reported to increase in frequency with age [6].

Large regenerative hepatic nodules are observed in up to 30% of ALGS patients and are postulated to occur in response to abnormal vascular perfusion of the liver [7, 8]. These lesions are typically solitary and centrally located close to the right portal vein. On radiological evaluation, these lesions have normal hepatic vessels and are relatively stable on follow-up imaging studies, with a mean interval of 33 months [8]. There is general consensus that the mechanisms leading to hepatic nodules in ALGS are reactive in nature as opposed to neoplastic.

Hepatocellular carcinoma (HCC) is considered a rare complication of ALGS. A recent case series and review of the literature described 21 children with ALGS who developed HCC [9]. The median age of HCC diagnoses was 4 years old (range 1–16 years), with the youngest case reported being a 1.5-year-old child. Cirrhosis was reported in all cases except for one at the time of HCC diagnosis. Although ALGS patients are not considered a high-risk group for HCC, monitoring of serum alpha-fetoprotein levels and an ultrasound examination is recommended every 12 months.

There is considerable variation in the clinical course and ultimate prognosis of liver disease in ALGS. For those children with significant hepatic involvement in infancy, the clinical picture is dominated by cholestasis and follows a severe course in the first 5 years of life, after which it appears to improve for many patients. This spontaneous improvement is poorly understood but well documented. In approximately 10–20%, the cholestasis persists unabated or progresses to end-stage liver disease (ESLD) with the onset of portal hypertension later in childhood. It is difficult to predict early on which ALGS children with cholestasis in early childhood will eventually require liver transplantation (LT) and which will spontaneously improve. There are no known genotypic or radiologic predictors of liver disease progression in ALGS. A review of laboratory data of ALGS patients showed that bilirubin and cholesterol levels before the age of 5 may aid in distinguishing patients at high and low risk of problematic cholestasis in later childhood. More specifically, mean levels of total bilirubin (TB) >6.5 mg/dL (111μmol/L), conjugated bilirubin (CB) >4.5 mg/dL (77μmol/L), and cholesterol >520 mg/dL (13.3 mmol/L) are strongly associated with severe liver disease in later life, whereas levels lower than this are associated with a more favorable hepatic

outcome [10]. These data may assist the clinician in predicting which children might go on to resolve their cholestasis and therefore avoid unnecessary LT in young children with ALGS.

The overall hepatic prognosis in ALGS was previously regarded as favorable, with reportedly only 20–30% requiring LT [6, 11]. However, these data represent mixed cohorts of children with ALGS and those with and without significant liver disease. Recently, Kamath et al. recently reported on the outcomes of 293 ALGS patients with cholestasis in a prospective observational multicenter North American study [12]. Native liver survival in ALGS probands with cholestasis was only 24% at 18.5 years. As described above, in early childhood, LT in ALGS typically occurs due to complications of cholestasis, and this study revealed an additional burden of liver disease in later childhood due to fibrosis and portal hypertension [6, 11, 13].

Cardiac

Cardiac anomalies are a hallmark feature of ALGS, with a reported prevalence of 94% [14]. The extent and pattern of cardiac involvement is highly variable, ranging from an asymptomatic murmur or peripheral pulmonary artery stenosis (PPS) to more complex intracardiac anomalies such as tetralogy of Fallot with or without pulmonary atresia. PSS accounts for approximately 73% of reported cardiac anomalies in ALGS and can lead to pressure or volume overload of the right ventricle (RV), RV hypertrophy, and, in severe cases, right-sided heart failure. The most common serious intracardiac defect is tetralogy of Fallot (TOF), which occurs in 7–12% [6, 14]. Approximately 40% of patients with ALGS demonstrating TOF have pulmonary atresia, representing a more severe phenotype. Cardiac disease accounts for nearly all of the early deaths in ALGS. Patients with intracardiac disease have approximately a 40% rate of survival to 6 years of life, compared with a 95% survival rate in patients with ALGS without intracardiac lesions [6].

Characteristic Facies

Individuals with ALGS often have a characteristic facial appearance that may include a high forehead with frontal bossing, deep-set eyes with moderate hypertelorism, saddle or straight nose with a bulbous tip, and/or pointed chin (Fig. 62.2). Together, these features give the face an inverted triangle appearance. This facial appearance is one of the most penetrant findings in ALGS and is reported in up to 90% of patients with a *JAG1* variant [6]. In early infancy, characteristic ALGS facial features may be difficult to recognize; however, this feature becomes increasingly evident

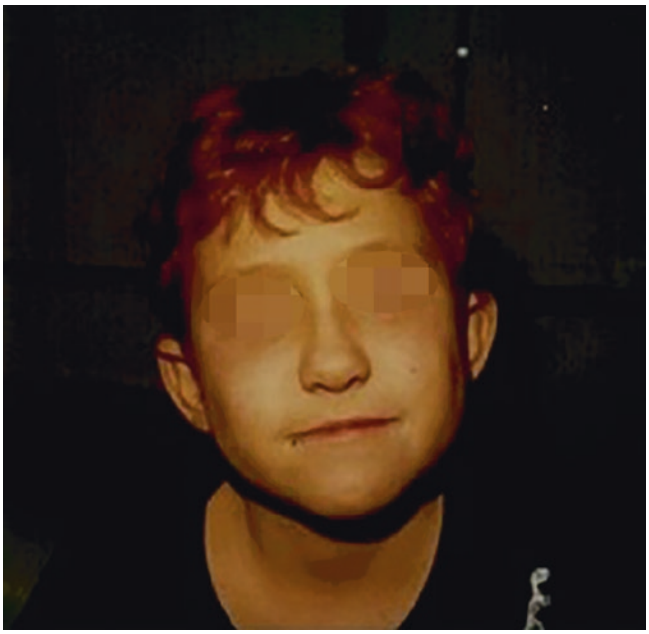

Fig. 62.2 Facial features of Alagille syndrome

with age. In one study, a series of photographs from patients with ALGS and patients with other known early-onset liver diseases were evaluated by clinical dysmorphologists to determine the diagnostic sensitivity and specificity of ALGS facies. The study authors found clinical dysmorphologists were able to correctly identify ALGS facial features in 79% of pediatric cases [15]. Interestingly, in adults, ALGS facial features were more difficult to identify and were correctly identified in only 67% of cases. The author's clinical experience is consistent with this finding, which suggests the forehead becomes less prominent after puberty, while the protruding chin is more noticeable, thus losing the overall triangle appearance. Recognition of ALGS-specific facies may aid in diagnoses, particularly in adult ALGS patients who present with idiopathic cardiac or renal disease and little or no hepatic involvement.

It should be noted that the presence of ALGS facial features might be less evident among individuals with ALGS and Vietnamese heritage [16]. When evaluated by North American dysmorphologists, characteristic ALGS facies was only identified correctly in 24% of Vietnamese children with ALGS and a *JAG1* variant. A higher sensitivity was reported by the referring pediatric gastroenterologist in Vietnam, who identified ALGS facies in 61% of children. Furthermore, a notably lower incidence of characteristic facial features has been described in ALGS patients with a *NOTCH2* variant (20%) in comparison to those with a *JAG1* variant (79%) [17]. These findings were recently confirmed in a congress abstract by the Global Alagille Alliance (GALA) Study Group, which describes the largest cohort of *NOTCH2*-associated ALGS to date [18]. These data indicate that

clinicians should evaluate these patient populations for other extrahepatic involvement instead of relying on the characteristic facies.

Ophthalmologic

The ocular abnormalities of patients with ALGS do not generally affect vision but are important as diagnostic tools. A large and varied number of ocular abnormalities have been described, though posterior embryotoxon is the most important diagnostically. Posterior embryotoxon is a prominent, centrally positioned Schwalbe's ring (or line) at the point at which the corneal endothelium and the uveal trabecular meshwork join and is visible on slit-lamp examination. Posterior embryotoxon occurs in 56–88% of patients with ALGS and was also detected in 22% of children evaluated in a general ophthalmology clinic [19]. Posterior embryotoxon is seen in other multisystem disorders such as chromosome 22q deletion, as well. The Axenfeld anomaly, seen in 13% of patients with ALGS, is a prominent Schwalbe's ring with attached iris strands and is associated with glaucoma. Optic disk drusen identified using ocular ultrasonography has been described in ALGS patients with high prevalence, but this test is not routinely performed [20].

Skeletal Involvement

Vertebral abnormalities are described in the initial reports of this syndrome. The most characteristic finding is the sagittal cleft or butterfly vertebrae, which is found in 33–87% of patients with ALGS [1, 13, 21, 22]. This relatively uncommon anomaly may occur in normal individuals and is also seen in other multisystem abnormalities, such as 22q deletion syndrome and VATER (vertebral defects, anal atresia, tracheoesophageal fistula, radial and renal defect) syndrome. The affected vertebral bodies are split sagittally into paired hemivertebrae because of a failure of the fusion of the anterior arches of the vertebrae. The mildly affected vertebrae have a central lucency. A fully affected vertebra has a pair of separate triangular hemivertebrae whose apices face each other like the wings of a butterfly. Generally, these anomalies are asymptomatic and of no clinical significance. Other associated skeletal abnormalities include an abnormal narrowing of the adjusted interpedicular space in the lumbar spine, a pointed anterior process of C1, spina bifida occulta, fusion of the adjacent vertebrae, hemivertebrae, the absence of the 12th rib, and the presence of a bony connection between ribs. In addition, supernumerary digital flexion creases have been described in one-third of patients [23].

Severe metabolic bone disease with osteoporosis and pathologic fractures is common in patients with ALGS. Recurrent fractures, particularly of the femur, have been cited as an indication for LT. Preliminary survey data suggests that there is a propensity toward pathologic lower extremity long bone fractures in ALGS [24]. A number of factors may contribute to osteopenia and fractures, including severe chronic malnutrition, vitamin D and vitamin K deficiency, chronic hepatic, and renal disease. It is not yet known whether there is an intrinsic defect in cortical or trabecular structure of the bones in patients with ALGS. Olsen evaluated bone status in prepubertal children with ALGS and identified significant deficits in bone size and bone mass that were related to fat absorption but not dietary intake [25]. In a study from the Childhood Liver Disease Research Network (ChiLDReN), 49 ALGS patients and 99 children with other inherited chronic liver diseases underwent dual-energy X-ray absorptiometry (DXA) scans [26]. In ALGS, DXA measures were found to be low but improved after adjustment for weight and height. Of note, DXA Z-scores in the ALGS population correlated negatively with measures of cholestasis including TB and serum bile acid levels. These data support multifactorial influences on bone density in ALGS, with possible contribution of impaired Notch signaling.

ALGS patients are frequently found to have short stature, and this is likely multifactorial in origin, resulting from cholestasis and malabsorption, congenital heart disease, and genetic predisposition. A validated growth curve for ALGS individuals is not yet available.

Renal Involvement

Renal involvement in ALGS has been widely reported on an individual case basis or as part of a larger report on general features of ALGS. The prevalence of renal involvement in larger series ranges from 40% to 70% such that it has been proposed that renal anomalies now be considered a disease-defining criterion in ALGS. In a large retrospective study, there was a prevalence of 39% of renal anomalies or disease. The most common renal involvement was renal dysplasia (59%), followed by renal tubular acidosis (10%), vesicoureteric reflux (8%), and urinary obstruction (8%) [3]. Hypertension in patients with ALGS can be of cardiac, vascular, or renal etiology.

Functional and structural evaluation of the kidneys should be undertaken in all patients. Renal function should be reassessed during the evaluation for LT (see Management below).

Vascular Involvement

Cerebrovascular anomalies are a well-known feature of ALGS and are a significant contributor to morbidity and

early mortality [2]. Three distinct clinical phenotypes have emerged in ALGS, including (1) cerebral aneurysms, (2) syndromic moyamoya, and (3) anomalies of the internal carotid artery. The main clinical complication of these anomalies in ALGS is stroke, either ischemic or hemorrhagic. Ischemic stroke is typically associated with moyamoya, while cerebral aneurysms represent the most common cause of hemorrhagic stroke among patients with ALGS. The presence of a vasculopathies in ALGS is consistent with the intrinsic role of the Notch signaling pathway in vascular formation and morphogenesis.

Retrospective studies in small cohorts of ALGS patients have reported wide ranges for the prevalence of cerebrovascular anomalies [2]. In one study, Emerick et al. retrospectively reviewed neuroimaging reports in 26 children with ALGS and identified anomalies in 38% of patients [27]. In this series, 100% of symptomatic ALGS patients and 23% of clinically asymptomatic patients had vascular anomalies detected by imaging studies. However, most concerning, cerebral angiography failed to detect any vascular anomaly in two asymptomatic patients, who both subsequently suffered fatal intracranial events several years after their baseline screening. These findings suggest the progression of underlying cerebrovascular disease. A more recent retrospective analysis by Carpenter et al. evaluated arterial and venous abnormalities in 19 children and young adults with ALGS [28]. The study authors reported cerebral arterial disease in 32% ($N = 6/19$) of ALGS patients, while venous anomalies were present in 21% ($N = 4/19$). Like Emerick et al., most patients were clinically asymptomatic at the time of neurovascular imaging, suggesting a high prevalence of silent cerebral vasculopathies.

It is now recognized that vasculopathies in ALGS extend beyond the central nervous system (CNS). Reported systemic vascular abnormalities include aortic aneurysms and coarctations and celiac, hepatic, and renal arterial anomalies. In a large retrospective chart review of 268 patients with ALGS, 25 patients had vascular complications (9%), including three patients with aortic aneurysms, two with aortic coarctations, and one patient with bilateral renal artery stenosis [2]. The frequency of intra-abdominal vascular anomalies has particular significance for LT in ALGS [29].

Bleeding Tendency in ALGS

Intracranial bleeding events are a concerning and potentially life-threatening complication of ALGS. Clinical series have reported cerebrovascular events in up to 16% of ALGS patients, with 30–50% of events resulting in mortality [2, 21]. Bleeding events in ALGS do not follow a distinct or predictable pattern and may occur spontaneously or with minimal trauma. An increased susceptibility to systemic bleeding events has also been observed in ALGS and appears to be unrelated to coagulopathy. In a retrospective chart review of 174 ALGS patients, 22% ($n = 38/174$) of individuals had one or more bleeding episodes, in the absence of coagulopathy [30]. The majority of the reported bleeding events occurred spontaneously or during a surgical or other invasive procedure. Concerningly, bleeding events accounted for 14% of deaths in the cohort.

The underlying processes responsible for the heightened risk of spontaneous bleeding, particularly intracranial bleeding, among individuals with ALGS remain unclear. Impaired integrity of blood vessels due to an underlying developmental defect, particularly within the CNS, has been cited as a possible explanation. Moreover, as discussed in the preceding section, underlying cerebral vascular anomalies, including cerebral aneurysms, are known to contribute to some cases [31–33].

Infections and Immune Dysregulation

It has been suggested that some individuals with ALGS may exhibit an immune-deficient phenotype characterized by recurrent episodes of otitis media with effusion (OME) and upper respiratory tract infections (URTIs). Serious, recurrent bacterial infections have not been reported in ALGS. Recurrent OME and URTIs have been described in up to 35% of children with ALGS, and it is hypothesized that this stems from an intrinsic defect in Notch signaling, specifically in CD46-Jagged1 interactions [13, 34]. CD46 is a complement and immune regulator that plays a critical role in mediating NOTCH expression during T cell activation. CD46 also mediates innate and adaptive immune responses. In a small case series, Shamouna et al. demonstrated increased Jagged1 expression on resting T cells in four immune-deficient ALGS patients. Furthermore, CD4+ T cells in these patients failed to elicit an efficient Th1 response to sustain induction of interferon (IFN)-γ production, both in vitro and in vivo. Similar findings have been described in CD46-deficient patients. The study authors also speculate that the molecular mechanisms leading to immune defects in ALGS may predispose patients to asthma, eczema, and food allergies [35, 36]. However, further studies are needed to understand how dysfunction in Notch signaling contributes to immune dysregulation in children with ALGS and the clinical significance of this problem.

Hearing Loss

Children with ALGS are at greater risk for both conductive and sensorineural hearing loss (SNHL). ALGS-specific features may be a contributing factor to OME (as described

above) and conductive hearing loss. Structural anomalies in the middle and inner ear have been described in ALGS, including partial or a complete absence of the bony and membranous structures of the posterior semicircular canals and hypoplasia of the anterior semicircular canals [37–39]. In one series, Teng and colleagues conducted hearing tests in 44 children with ALGS, of whom 13 had hearing loss in at least one ear [40]. Conductive hearing loss (27% of left ears, 30% of right ears) was the most common hearing impairment, followed by mixed hearing loss (14% of left ears, 9% of right ears) and SNHL (4% of left ears, 11% of right ears). More recently, in large multicenter cohort, Kamath et al. investigated audiological manifestations in 110 children with ALGS and cholestatic liver disease. Air conduction testing was performed and revealed 38% (n = 42) of participations failed at least one frequency in one or both ears [12]. Tympanometry was subsequently performed in those who failed the air conduction test and identified 67% (n = 28/42) of participants failed in one or both ears. These data indicate a high prevalence of hearing impairment among ALGS patients and the need for ongoing surveillance and hearing screening.

Arthritis

The number of reported cases of arthritis in ALGS has increased in recent years and may be more common than initially appreciated. In 1993, Jacobs and colleagues reported the first case of a child with ALGS and severe, refractory juvenile idiopathic arthritis (JIA) [41]. Two subsequent case reports have also described JIA in a child and an adult with ALGS [42, 43]. More recently, in an international, multicenter study, Ferrara et al. described ten children with ALGS and features of JIA [44]. The median age of onset was 6.5 years (range 2–10 years), and the median number of joints with active arthritis was 2 (IQR 0–4). The most commonly affected joints were the knees (90%) and ankles (70%), followed by the small joints of the hands or feet (50%). Notably, only 33% (n = 2/6) of ALGS children had positive antinuclear antibody (ANA) titers. The study authors pooled together all ALGS cases from their seven centers with and without JIA and reported a combined prevalence rate of 5% (n = 10/195). Strikingly, the estimated prevalence rate of JIA is 1 in 1000 children (<0.1%), suggesting that children with ALGS have a 50-fold increased risk of developing arthritis compared to the general pediatric population [44, 45].

The underlying mechanisms involved in the pathogenesis of arthritis in ALGS patients remain elusive. However, a growing body of evidence has demonstrated that Notch signaling is activated in autoimmune and inflammatory-related diseases, such as rheumatoid arthritis (RA) and osteoarthritis [46]. For instance, Jiao and colleagues found that Th cells in individuals with active RA expressed significantly higher levels of Notch2, Notch3, and Notch4 at the mRNA and protein level in comparison to health controls [47]. Further evidence demonstrating a link between the Notch pathway and inflammation is shown by Park et al., who found pharmacologic inhibition of Notch signaling modulates disease activity in experimental inflammatory arthritis [48]. Collectively, these studies suggest that mutations in genes encoding components of the Notch signaling pathway, including *JAG1* and *NOTCH2*, could lead to arthritis in ALGS. Future clinical studies will be necessary to determine whether the type of arthritis observed in ALGS is similar to JIA or is an extension of the ALGS phenotype.

Genetics of ALGS

ALGS is inherited in an autosomal dominant manner, with highly variable expressivity. It is a genetically heterogeneous disorder and may be caused by mutations in either *JAG1* (seen in 94% of clinically defined probands) or *NOTCH2* (seen in approximately 2.5%) [4, 17, 49, 50]. Jagged1 is a cell surface protein that serves as a ligand for the four Notch receptors (Notch1, 2, 3, and 4), and together these proteins begin the cascade of events that turn on the Notch signaling pathway. The Notch signaling pathway is involved in the determination of cell fate and as such plays a crucial role in normal development.

Gene Identification and Mutation Analysis

JAG1 was identified as the cause of ALGS in 1997 [49]. To date, more than 430 *JAG1* mutations have been identified in patients with ALGS. Utilizing current screening techniques, the mutation detection rate is 94% [4]. The frequency of sporadic mutations (i.e., new in the proband) is approximately 60–70%. Approximately, 75% of ALGS patients have *JAG1* protein-truncating (frameshift or nonsense or splice-site) mutations [4]. Approximately 7% have gene deletions. Missense mutations are identified in 15%. Haploinsufficiency, a decrease in the amount of the normal protein, is hypothesized to be the mechanism causing ALGS. ALGS associated with *NOTCH2* mutations was described in 2006. Thus far, 23 patients with unique *NOTCH2* mutations have been described [4, 17, 50].

A small fraction (3–5%) of ALGS individuals have deletions of chromosome 20p. Genome-wide single-nucleotide polymorphism (SNP) analysis of 25 patients with ALGS revealed 21 deletions ranging from 95 kb to 14.62 Mb [51]. Patients with deletions greater than a critical 5.4 Mb region had additional phenotypic features not usually associated with ALGS such as developmental delay and hearing loss.

Genotype–Phenotype Correlations

JAG1 Mutations

Although the ALGS phenotype is highly variable, there is no apparent correlation with *JAG1* genotype in the majority of patients. A study of 53 *JAG1* mutation-positive relatives of a cohort of ALGS probands demonstrated that only 53% met the clinical criteria for a diagnosis of ALGS, including 11 of 53 with obvious clinical features that would easily have led to a diagnosis of ALGS and 17 of 53 (32%) who had mild features that would have only been apparent on targeted evaluation following the diagnosis of a proband in their family (i.e., discovery of elevation of liver enzymes or posterior embryotoxon in an asymptomatic individual) [52]. This underscores the variable clinical consequences associated with a *JAG1* mutation and suggests the presence of genetic modifiers.

NOTCH2 Mutations

While the phenotype of *JAG1*-associated ALGS has been reasonably well characterized, *NOTCH2*-associated disease has only been reported in a small number of individuals. Pathogenic or likely pathogenic *NOTCH2* variants have been reported in 23 children with ALGS with complete or partial features of the classic phenotype [4]. Recently, in a congress abstract, the GALA Study Group described 19 ALGS patients with *NOTCH2*-associated disease, the largest comprehensive analysis to date. In comparison to *JAG1*-ALGS patients ($N = 755$), *NOTCH2*-ALGS were significantly less likely to have characteristic facies (53% vs. 88%, $p < 0.001$), an ECHO-confirmed cardiac anomaly (58% vs. 93%, $p = 0.001$), posterior embryotoxon (14% vs. 53%, $p = 0.004$), or butterfly vertebrae (0% vs. 44%, $p = 0.001$) [18]. Notably, 10- and 18-year native liver survival rates in patients presenting with neonatal cholestasis were comparable between the two variant groups (59% and 74%; 48% and 62%, respectively; log-rank $p = 0.31$). These data suggest that reliance on classical clinical phenotypic definitions of ALGS may miss patients with *NOTCH2*-related disease and that genetic testing is crucial for diagnosis.

Diagnostic Considerations

Clinical Diagnostics

The majority of infants with ALGS are evaluated for conjugated hyperbilirubinemia in the first weeks or months of life. ALGS is occasionally misdiagnosed as BA because of the overlap of biochemical, scintigraphic, histologic, and cholangiographic features. Serum bilirubin, bile acid, and GGT levels typically are elevated in both of these disorders. Ultrasound findings in both conditions may reveal small or apparently absent gallbladders. Excretion of nuclear tracer (diisopropylacetanilido iminodiacetic acid, DISIDA) into the duodenum excludes BA, but non-excretion of tracer is also possible in ALGS. There was no excretion of scintiscan in 61% of 36 infants with ALGS [6].

Although a liver biopsy is not mandatory to diagnose ALGS, it remains an important step in differentiating between ALGS and BA in infants with high-GGT cholestasis. In BA, bile duct proliferation is the typical histologic lesion. In ALGS, paucity is evident in 60% of infants younger than 6 months but in 95% of older patients [6]. Unfortunately, there may be a normal number of ducts early in the course of BA and also in some patients with ALGS, and bile duct proliferation occasionally occurs in infants with ALGS. Giant cell hepatitis is also seen in both disorders. Finally, it should be noted that bile duct paucity, if present, is not diagnostic of ALGS and other diagnoses should be considered (e.g., alpha-1-antitrypsin deficiency, cystic fibrosis, cytomegalovirus infection, etc.).

An operative cholangiogram is considered the gold standard procedure to evaluate the extrahepatic and intrahepatic biliary tree; however, this can also be misleading in ALGS. The extra- and intrahepatic ducts are extremely small in patients with ALGS, and the cholangiogram commonly does not demonstrate communication proximally. In 37% of 19 cholangiograms in infants with ALGS, there was no opacification of the proximal extrahepatic ducts, and, in another 37%, the proximal extrahepatic tree was abnormally small [6]. The intrahepatic ducts were normal in only 10% of 19 infants with ALGS, small or hypoplastic in 16%, and not visualized in 74%. Therefore, even the apparent gold standard test to differentiate ALGS and BA can be misleading.

Clinical features in extrahepatic organ systems may help in the diagnostic evaluation. The list of abnormalities identified in the "major" organ systems and the list of other affected organs have grown appreciably. Thus, an echocardiogram, slit-lamp examination, renal ultrasound, and spinal X-ray are essential diagnostic tests when ALGS is suspected. It should be noted that several of the ALGS-defining features are present in normal individuals or other conditions. Heart murmurs are present in 6% of all newborns, posterior embryotoxon appears in 22% of the general population, and butterfly vertebrae are seen in 11% of patients with 22q11 deletion. Furthermore, the facial features of ALGS patients are subtle during the first months of life making this an unreliable diagnostic tool in infancy.

With the advent of molecular testing for ALGS and the broader appreciation of the phenotypic variability, the diagnostic criteria for ALGS have been modified. To make a

clinical diagnosis for an index case (proband) in the family, the original Alagille criteria hold, modified only so as no longer to require histology and expanded to include renal and vascular involvement. Thus, ALGS can be diagnosed clinically on the basis of cholestasis with at least three features from the list of characteristic Alagille facies, consistent cardiac disease, posterior embryotoxon, butterfly vertebrae, typical ALGS renal disease, and a structural vascular anomaly. In families with one definite clinically defined proband, other members with only two features should be considered as having ALGS.

Molecular Diagnostics

Molecular diagnostics is now widely commercially available for *JAG1* and *NOTCH2*. For practical reasons and cost-effectiveness, simultaneous testing of both genes is now carried out by readily available next-generation sequencing (NGS) panels. A molecular diagnosis can assist in an atypical ALGS case and is also useful for genetic counseling and prenatal diagnosis. *JAG1* sequencing identifies mutations in individuals with clinically defined ALGS in the majority of cases (>95%) [4].

Once a *JAG1* mutation is identified in a proband, it is simple to test parents and other relatives for the identified mutation. Mutations are inherited from an affected parent in 30–50% of patients, whereas the mutations appear de novo in 60–70% [4]. If a parental mutation is identified, there is a 50% risk for each future offspring to inherit the *JAG1* mutation. However, it should be emphasized that expressivity of the disorder is highly variable, and it is not currently possible to predict disease severity in a new baby.

Prenatal genetic testing for ALGS is possible if a parental mutation has been identified. This requires amniocentesis or chorionic villous sampling and assessment for a known *JAG1* mutation. Preimplantation genetic diagnosis has also been successfully performed in ALGS. It is imperative to carefully counsel parents undergoing any type of prenatal testing since there are no genotype–phenotype correlations in ALGS, so it is not possible to make predictions about a child's clinical course based on the type or presence of a mutation.

Management

Management of Cholestasis

Patients with ALGS present significant management challenges due to profound cholestasis and complex multisystem disease [53]. A sequential and additive approach to medical cholestasis therapy in ALGS is most appropriate. The most commonly used agents are listed in Table 62.1 with common side effects and described below.

The pruritus associated with ALGS cholestatic liver disease can be severe and may occur even without jaundice. Pruritus is often debilitating, disturbing sleep, daily activities, and cognitive development. Conservative management of pruritus entails taking care to keep the skin hydrated with emollients, trimming the fingernails, and taking short baths or showers to limit drying of the skin. Bile flow may be stimulated with choleretics, and ursodiol is the most commonly used agent. The use of ursodiol has been studied in ALGS children with improvement in pruritus, xanthomas, and biochemical markers of cholestasis [12, 39–41]. Therapy with antihistamines may provide some symptomatic relief but is rarely effective alone. Bile-acid-binding resins, such as cholestyramine, can be effective but more often are not palatable. They are also difficult to administer since they must be given 2 h apart from other medications. Colesevelam may be better tolerated but has not been studied in pediatrics. It should be noted that colesevelam is a very potent bile-acid-binding resin and may severely deplete the concentration of free luminal bile acids resulting in risk of fat-soluble vitamin deficiency, and these levels should therefore be monitored. Rifampin has been comparatively well studied in ALGS [54, 55]. Yerushalmi et al. studied 24 children with severe cholestasis, of whom 6 had AGS, and 92% of the cohort showed a response in improving pruritus [55]. Although rifampin is associated with elevation of serum transaminases, none of these studies reported clinical or biochemical adverse events. Naltrexone has been shown to be effective against pruritus in cholestatic adults [56, 57]. Based on limited experience, it can be useful in the pruritus of ALGS children as well [58].

Table 62.1 Medications for cholestasis in Alagille syndrome

Medication	Dose	Most frequent side effects
Choleretics		
Ursodeoxycholic acid (Actigall)	10–20 mg/kg/day, divided in two doses	Diarrhea, abdominal pain
Others		
Rifampin	10 mg/kg/day, divided in two doses (maximum dose 600 mg/day)	Red discoloration of urine, idiosyncratic hypersensitivity, reactions, hepatitis
Sertraline (Zoloft)	75–100 mg/day (maximum dose of 200 mg/daily)	Restlessness, loss of appetite
Naltrexone	1–2 mg/kg orally once daily (maximum dose of 50 mg/day)	Nausea, abdominal pain
Bile-salt binding agents		
Cholestyramine	240 mg/kg/day, divided into three doses; maximum dose of 8 g/day	Constipation, abdominal pain

Emerging data also exist for the use of sertraline in cholestatic pruritus, but its use is not widespread [59].

Newer agents that inhibit apical sodium-dependent transport of bile acids (ASBT inhibitors or inhibitors of bile acid transport (IBAT)) act at the enterocyte and interrupt the enterohepatic circulation. These drugs reduce the total bile acid pool size, effecting a chemical biliary diversion [60, 61]. Currently, two drugs in this class are under study for pruritus in children with cholestasis, maralixibat and odevixibat. At this time, there are more data available for maralixibat in ALGS. Maralixibat has been evaluated in phase 2 trials in patients with ALGS. The ITCH trial evaluated 37 patients with ALGS in a placebo-controlled randomized trial [62]. Although the prespecified primary endpoints were not met in this study, a reduction in pruritus, as measured by caregivers via the validated itch-reported outcome instrument (ItchRO), was more common in the maralixibat-treated group as compared to the placebo group. Maralixibat was well tolerated with comparable adverse events between groups. The ICONIC trial evaluated 31 patients with ALGS in a multicentered trial using a randomized drug-withdrawal study design (these data have only been presented in abstract form, to date) [63]. Serum bile acid levels and pruritus scores fell on maralixibat treatment; however, during the randomized drug-withdrawal period, bile acid levels in the placebo group returned to baseline, and subjects had significantly higher pruritus scores with improvement in both of these parameters on restarting the drug [63]. These preliminary studies show that IBAT inhibitors hold promise as future treatments for pruritus that may potentially also prove to be hepatoprotective.

Surgical biliary diversion has been successful in a number of patients, though it does not appear to be as effective for ALGS as it is for progressive familial intrahepatic cholestasis (PFIC) [64–66]. Wang et al. evaluated the efficacy of non-transplant surgical interventions in 58 children with severe cholestasis, including 20 participants with ALGS [67]. The median age of surgical diversion was 30 months (Q1/Q3 = 26/86 months, range = 2–218 months, mean = 65 months), and the leading indication was severe pruritus (100%), followed by xanthomata (50%) and progression of liver disease (25%). Among the ALGS patients, 15 underwent partial external biliary diversion (PEBD), which led to a significant decrease in total serum cholesterol (695 ± 465 vs. 457 ± 319 mg/dL; preop vs. 12–24 months postop, respectively; $p = 0.0001$). Surprisingly though, there was not a significant decrease in serum TB (ALGS: 0 = 5.3 ± 5.4, 24 mos. = 4.9 ± 4.1 mg/dL). Ileal exclusion (IE) was performed in five ALGS patients, and none of these patients experienced relief of their xanthomata over 24 months postoperatively. External biliary diversion appears to be more effective than internal approaches though the impact on quality of life of a stoma limits the acceptability of this approach with many families.

Hepatoportoenterostomy is not indicated in children with ALGS and is associated with adverse clinical outcomes. In a limited retrospective study comparing ALGS patients to matched BA patients after Kasai procedures, the ALGS cohort had a significantly higher rate of LT (47 vs. 14%) and sustained higher mortality [68]. These data suggest that the Kasai procedure is not a marker for severe underlying liver disease but that the Kasai procedure itself has a detrimental effect on outcome. Similar negative outcomes were observed in a recent systematic review and meta-analysis [69].

Xanthomas do not require specific treatment unless they interfere with vision, feeding, or motor development which is exceptionally rare. As the hypercholesterolemia of ALGS is not atherogenic, dietary modifications or medical therapy is not necessary.

Nutritional

Failure to thrive and malnutrition need to be addressed aggressively and early on in life. There is significant malabsorption of long-chain fat; therefore, formulas supplemented with medium-chain triglycerides may have some nutritional advantage. Many patients are unable to eat enough to provide the substantial quantities of energy required for growth and development, and nasogastric or gastrostomy tube feedings can provide necessary supplementation.

Fat-soluble vitamin deficiency is present in most patients with significant ALGS cholestasis. Oral or parenteral supplementation is necessary for prevention of vitamin deficiencies and their sequelae. Standard multivitamin preparations may not provide the correct ratio of fat-soluble vitamins, unless they have been specifically formulated for cholestasis (e.g., DEKAs). If such cholestasis-specific preparations are not available, vitamins are best administered as individual supplements tailored to the specific needs of the patient. Close monitoring (every 3–6 months) of fat-soluble vitamin levels is crucial to avoid complications of vitamin deficiencies, particularly in the first years of life.

Liver Transplantation

LT is an effective therapeutic modality for ALGS patients and is indicated in 20–50% of all pediatric cases, and this varies according the heterogeneity of the cohort being reported. Clinical indications for LT in ALGS can be categorized into two groups: (1) those with one or a combination of complications related to chronic cholestasis, including refractory pruritus and disfiguring xanthomas, failure to thrive, fat-soluble vitamin deficiency, and/or recurrent pathological fractures, and (2) those with biliary cirrhosis and/or evidence of portal hypertension. The burden of extrahepatic comorbidities in

ALGS, including cardiac, renal, and vascular involvement, is important determinant of transplant outcomes in terms of both morbidity and mortality. As such, a multifaceted, multidisciplinary team-based approach is recommended.

A thorough and in-depth assessment of the cardiovascular system is a key part of the transplant candidate evaluation in children with ALGS, and special attention should be paid to the functional capacity of the right ventricle. Cardiac evaluations should include a chest X-ray, electrocardiogram (ECG), and two-dimensional echocardiogram (ECHO). However, a limitation of these testing modalities is they can underappreciate the peripheral branches of the pulmonary arteries and the degree of right ventricular hypertrophy in this population. Therefore, the King's College Group suggests a cardiac catheterization and dynamic stress test with dobutamine (DSE) to simulate perioperative conditions in the LT evaluation of ALGS patients [70]. This approach induces peripheral vasodilatation and increases cardiac output, thereby mirroring the conditions of graft reperfusion. If the transplant candidate achieves a cardiac output of more than a 40% during DSE, the patient's cardiac reserve is considered sufficient for transplantation.

Given the high rate of renal involvement in ALGS, a detailed evaluation of the kidneys is indicated in all transplant candidates, including a measured glomerular filtration rate (GFR) and in some institutions serum cystatin C. Furthermore, to prevent subsequent renal injury during the posttransplant course, the authors advocate for the use of a renal-sparing protocol in all ALGS transplant recipients, regardless of pretransplant renal function.

Living-related liver transplantation (LRLT) in ALGS requires careful thought and consideration. Although 60% of ALGS cases result from de novo mutations, the possibility of a first-degree relative harboring the same pathogenic variant as the proband needs to be entertained. Therefore, targeted genetic testing of *JAG1* and *NOTCH2* is recommended for any biological relatives of an ALGS patient considering becoming a living-related donor. Indications for LT in ALGS are not typically emergent, and the time scale for predictive genetic testing for prospective donors is rapid, approximately 2–4 weeks. On a cautionary note, Gurkan reported two instances in which apparently unaffected parents underwent donor operations that were unsuccessful because of bile duct paucity discovered intraoperatively [71]. These case examples underscore the importance of genetic testing in all. Therefore, the recommendation in North America is for potential donors to undergo screening for the known mutation in the proband and for them generally not to be used as donors if positive for the mutation.

Liver Transplant Outcomes

Overall patient and graft survival rates for children with ALGS are now comparable with other indications for pediatric LT [72, 73]. Marked improvements in survival rates over the past decade are attributed to careful candidate selection, better perioperative management of systemic disease features, and revised immunosuppression protocols.

In the largest series to date, Arnon and colleagues analyzed outcomes of 461 children with ALGS over a 21-year period [74]. They reported 1- and 5-year patient survival rates of 83% and 78.4% and 1- and 5-year graft survival rates of 75% and 62%, respectively. The study authors also compared posttransplant outcomes between children with ALGS and those with BA. Both 1- and 5-year patient and graft survival rates were lower in ALGS patients in comparison to BA (83% and 78% patient survival, 83% and 70% graft survival, respectively). Reduced posttransplant survival in ALGS may be partially explained by the multisystem nature of the syndrome and a greater burden of comorbidities. However, the United Network for Organ Sharing (UNOS) database does not capture characteristic features of ALGS such as cardiac, renal, or vascular involvement, limiting further interpretation of these findings.

A concerning observation among several large ALGS series is the high incidence of early posttransplant mortality (<30 days post-LT). In the previously described UNOS study by Arnon et al., pediatric LT recipients with ALGS were far more likely to experience early death in comparison to children with BA (9.6% vs. 4.8%, $P = 0.01$) [29]. Recipient characteristics associated with early mortality included higher mean pretransplant creatinine levels, cold ischemic time >12 h, and prior transplantation. A clustering of deaths in the immediate posttransplant period was also demonstrated in another Alagille-transplant study [73]. In contrast, the study authors did not identify any pretransplant variables associated with early mortality. However, complications, including biliary, vascular, renal, and CNS during the first 6 months following LT, were proportionally higher among children with ALGS, and these were prognostic markers for increased mortality.

Cardiac Anomalies

Management of the cardiac involvement in ALGS is clearly lesion specific. In a large, though now older, ALGS series, cardiac surgery was performed in infancy in 11% [6]. The mortality rates were 33% for those with TOF and 75% for those with TOF with pulmonary atresia. The survival of patients with ALGS with these lesions is markedly lower than for patients (with these lesions) without ALGS. This may be a result, in part, of the common presence of significant stenoses in the distal pulmonary artery or other systemic manifestations of the syndrome. Nonsurgical invasive techniques have been used successfully for patients with ALGS, including valvuloplasty, balloon dilatation, and stent implantation. Heart–lung transplantation has been performed in

combination with LT in a child with ALGS as a rare occurrence [75].

Other Extrahepatic Diseases

As with the cardiac involvement in ALGS, other extrahepatic diseases must be managed according to standard of care. Currently, there are no established guidelines for the screening and management of cerebral vasculopathies in ALGS, and prospective studies are lacking. Considering the potentially fatal consequences of cerebrovascular abnormalities, the authors recommend all individuals with ALGS undergone a screening MRI/MRA at approximately 8 years of age, when sedation is no longer required. Furthermore, children with ALGS need to be managed with a heightened sense of awareness and urgency in the setting of acute head trauma or any new neurologic signs of symptoms. In the setting of any major surgery, such as LT, all children with ALGS should undergo an MRI/MRA of the head and vascular imaging of the abdomen, typically CT angiography to identify vascular anomalies.

Prognosis of Alagille Syndrome

Overall mortality is difficult to ascertain in ALGS since the organ involvement is variable and so reported mortality rates reflect the nature of the underlying cohort. Cardiac, hepatic, and vascular diseases account for the majority of deaths in ALGS. The presence of a complex intracardiac anomaly is the only predictor of an excessive early mortality rate, and cardiac anomalies account for the majority of deaths in early childhood. Overall, vascular and bleeding events accounted for most of the mortality in one large series of ALGS: 34% [2]. Quiros-Tejeira reported a 72% survival rate in 43 patients at a mean follow-up of 8.9 years in a population in which 47% underwent LT [13]. Emerick estimated the 20-year survival rate in 92 patients to be 75% overall, 80% for those not requiring LT, and 60% for those requiring transplantation [6]. However, both of these case series are more than 20 years old. In the most recent assessment of outcomes in a prospectively followed cholestatic cohort of 293 ALGS patients, native liver survival was only 24% at 18.5 years, and during the study period only 11 patients died [12].

Conclusion

To conclude, ALGS is a complex condition in which the molecular basis is well understood, but the absence of identified genotype–phenotype correlations and the broad variability poses management challenges. Renal and vascular involvement should be included in the diagnostic criteria. The discovery of two disease genes and a broader phenotype, which includes individuals with *no* liver disease, suggests that a redefinition of this syndrome is warranted based on molecular defects, possibly reserving the term ALGS for those with liver disease and associated features, as Daniel Alagille originally intended. The most exciting management advance in ALGS is the promise of IBAT inhibitors which will likely revolutionize the treatment of pruritus and hopefully reduce the need for surgical biliary diversion and perhaps even LT.

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Chronic Viral Hepatitis B and C

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Abbreviations

ALT	Alanine aminotransferase
Anti-HBc IgG	Immune globulin G
Anti-HBc IgG	Immune globulin G
Anti-HBc	Antibody against HBcAg
Anti-HBc	Antibody against HBcAg
Anti-HBc	Antibody against HBcAg
Anti-HBe	Antibody against HBeAg
Anti-HBs	Antibody against HBsAg
Anti-HDV	Antibody against delta virus
cccDNA	Covalently closed circular DNA
DAA	Direct-acting antiviral
DNA	Deoxyribonucleic acid
EMA	European Medicines Agency
FDA	Food and Drug Administration
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immune deficiency virus
IgG	Immune globulin G
IL2	Interleukin 2
IL28B	Interferon lambda 3 gene
NCTP	Sodium/taurocholate cotransporting polypeptide
ORF	Open reading frame
RNA	Ribonucleic acid
SVR	Sustained viral response
TNF-α	Tumor necrosis factor alpha

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Chronic Hepatitis B

Introduction

Hepatitis B virus (HBV) infection remains a global health burden with estimated 250 million people chronically infected worldwide. Nevertheless, since HBV vaccine has become available for more than 25 years and many countries introduced vaccination programs as a prevention strategy on a regular basis for young infants, significant reduction of the incidence of acute hepatitis B in children and adolescents has been observed. Unprotected, approximately 90% of HBV-infected infants and 20–25% of those infected in preschool age will develop chronic infection decreasing to a chronicity rate of around 5% for adolescents and adults [1–3]. Despite a rather benign spontaneous course of the disease during childhood and adolescence, there is a considerable lifetime risk of progressive liver disease, liver cirrhosis, and the development of a hepatocellular carcinoma (HCC), which may eventually reduce life expectancy. Thus, careful long-term monitoring has to be performed, and appropriate treatment options, which unfortunately are not entirely curative at present, have to be considered.

Pathogenesis of Chronic HBV Infection

HBV belongs to a DNA virus family called hepadnaviruses. It contains a partially double-stranded DNA genome with about 3200 nucleotides. The minus strand covers four overlapping open reading frames (ORFs): S, for the surface gene encoding three envelope proteins (hepatitis B surface antigen, HBsAg); C, for the core gene encoding the core protein (hepatitis B core antigen, HBcAg); X, for the regulatory X gene; and P, for the polymerase gene encoding the viral DNA polymerase. By using multiple start codons, HBV is able to encode more than one protein from an ORF. After hepatocyte entry by the NCTP-receptor, the viral envelope is

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removed, and the nucleocapsid reaches the nucleus, where the double strand will be completed and converted into a covalently closed circular DNA (cccDNA). This is an important step, because the majority of cccDNA is then organized into nucleosomes forming the viral minichromosome, which is serving as template for the synthesis of the viral mRNA. The transcripts are translated into the viral proteins, and simultaneously reverse transcription leads to the synthesis of a complete minus strand of HBV DNA. The plus strand can then be synthesized again, and the molecule circularizes. Thus, the replication of HBV is similar to that of a retrovirus. The proteins are synthesized and assembled at the endoplasmatic reticulum and eventually discharged by vesicular transport as a Dane particle which contains the complete virus. The cccDNA plays a key role in viral persistence, viral reactivation after treatment withdrawal, and drug resistance. It accumulates in the nucleus of the hepatocyte as a stable minichromosome organized by histone and nonhistone viral and cellular proteins [4, 5]. Persistent HBV replication is associated with a high frequency of integration of HBV sequences into the human host liver cell genome. Enhanced DNA replication and DNA damage occurring during chronic inflammation with cycles of cell death and regeneration increase the availability of DNA ends in host genomic DNA and promote the process of viral integration [6]. Furthermore, it is presumed that certain altered cells are susceptible to the development of additional genetic and epigenetic changes that may lead to the development of malignant cell transformation and HCC.

For the understanding of the different phases during the course of the chronic disease, it is important to realize that the virus itself is not primarily pathogenic to the hepatocyte. The mechanism of cell death is generally accepted to be the result of a cytotoxic T-lymphocyte-mediated immune response of the host to the virus. Additionally, it has been shown that some HBV proteins may be able to induce apoptosis. During the transition from the immune-tolerant to the immune-active phase, a shift from the hepatitis e antigen (HBeAg)-specific Th2 cell tolerance to Th1 cell activation may recognize HBV-related epitopes on hepatocytes resulting in secretion of cytokines such as interleukin (IL)-2 and tumor necrosis factor alpha (TNF-α) and thus activating inflammation [7].

Epidemiology

Overall, the number of infected people is decreasing. Nevertheless, there are still high endemic countries in Asia, Africa, and some parts of South America with an HBsAg prevalence of more than 8%. The Arabian region, parts of the Eastern hemisphere, and Greenland show an HBV prevalence of 2–7%, and in the Western countries the rate is below 2% [8, 9]]. Global immunization programs have been established in many countries, and the HBV infection rate has declined worldwide. Vertical transmission has become the main route of infection; nevertheless, in some areas, HBV may also be a predominant disease in adolescents and adults due to high-risk sexual behavior and drug abuse [10]. Unfortunately, up to 2–15% of perinatal HBV infection of antibody against HBeAg (anti-HBe)- and HBeAg-positive mothers cannot be prevented by active and passive immunization due to intrauterine infection, vaccine failure, or HBsAg escape mutants [11, 12]. Thus, passive and active immunization has to be started immediately after birth in all newborns from HBsAg-positive mothers. HBeAg-positive mothers can be considered to receive treatment with the nucleoside analogues telbivudine and tenofovir in late pregnancy to decrease viral load [13, 14]. After complete immunization, there are no objections against breastfeeding. In countries with blood donor screening and serum testing, parenteral transmission does no longer play a significant role. The HBsAg prevalence in children is estimated between 0.02% and 0.03% in Western countries and the USA, 0.14% in Brazil, and 0.5% in Taiwan after immunization [1, 15]. Given HBsAg prevalence in pregnant women of 0.4% in Western Europe and an HBV transmission rate of 5–10% despite complete vaccination, 20–40 newborns in 100,000 births may be infected and become a chronic carrier state.

Ten HBV genotypes (A–J) have been documented showing a distinct distribution. Genotypes A and D are predominant in North America, Europe, and India, and genotypes B and C are mostly found in Asian countries. To date, routine determination of genotype is not yet recommended because treatment options are not adjusted to genotypes. Nevertheless, since there is line of evidence that genotypes C and D may be associated with more aggressive liver disease, this might become significant during the long-term follow-up [16].

Diagnostics

Chronic hepatitis B infection is defined as a repeatedly positive HBsAg test result within 6 months. Apart from the aminotransferases, HBeAg, anti-HBe, anti-HBcIgG, and quantitative HBV DNA have to be determined to confirm chronic hepatitis B and to classify the present stage. Additionally, antibody against delta virus (anti-HDV) should be tested to exclude concomitant hepatitis D. It is recommended to perform an ultrasound examination including liver stiffness assessment for baseline findings. Since chronic hepatitis B usually is a mild disease in terms of inflammation in childhood, histological examination by liver biopsy is not mandatory. However, in subjects who are suspicious of progressive liver disease or cirrhosis or if an impact on therapeutic decisions is identifiable, liver biopsy may be a reasonable completion.

Natural History

There are four natural stages of chronic hepatitis B infection: immune tolerance stage, immune reactive or immune clearance stage, inactive HBsAg carrier stage, and reactivation stage. The immune-tolerant phase is also named chronic hepatitis B infection and the immune-active phase chronic hepatitis B. As fifth and last stage, viral elimination with antibody against HBsAg (anti-HBs) seroconversion, which is a rather rare event occurring not more than 0.5% annually in children, could be denominated [7, 17]. Figure 63.1 illustrates the different phases of chronic hepatitis B.

Children who have HBV infection acquired perinatally or in the first months have an initial tolerance stage which is characterized by the presence of HBsAg, HBeAg, and extremely high HBV DNA levels (10^{7-9} virions/ml) and normal aminotransferases. The duration of the immune-tolerant phase is not predictable and may last 1–4 decades. Asian patients use to have a longer immune-tolerant stage. T helper (Th) cell immune tolerance is generated by HBeAg functioning as an immunoregulatory protein mostly already transplacentally transmitted. This kind of induced immune tolerance may explain the high chronicity rate and the younger the individuals are when they get infected. Usually, only minimal inflammatory activity is detectable in the liver tissue in this phase. Even in adult patients, no severe progression is expected during the immune-tolerant stage [18]. Although antiviral therapy is still not recommended for immune-tolerant subjects, they should be carefully monitored to duly recognize progression to immune-active phase.

With time, a nonspecific increase of inflammatory activity or a decrease of HBeAg serum concentration, which may be due to emerging mutants in the core promotor or precore region resulting in a lower HBeAg production, may activate HBeAg-specific T cell clones. In this immune reactive phase, HBeAg remains positive, and aminotransferases rise. HBV DNA remains high or stays at a little lower level. During this time, progression to liver fibrosis or cirrhosis may occur. However, liver cirrhosis rate is not expected to exceed 3–5% until reaching adulthood [19–21]. In the immune reactive phase treatment has to be considered.

A key event in the natural course of chronic hepatitis B is the HBeAg/anti-HBe seroconversion which occurs unpredictably for the single individual during the immune-active phase. Anti-HBe seroconversion is associated with a significant decrease of viral replication and normalization of aminotransferases reflecting the biochemical and histological remission of inflammatory activity. In some studies, the annual seroconversion rate depends on the route of infection and the ethnic origin. Whereas the mean seroconversion rate in non-Asian children ranges between 8% and 15%, seroconversion in Asian children was considerably lower with approximately 5% per year [7, 20]. Anti-HBe seroconversion is followed by the inactive HBsAg carrier state with persistently normal aminotransferases and a low viral load. Viral replication is considered low when HBV DNA serum concentrations remain below 2000 IU/ml. Some carriers may be lucky and develop anti-HBs antibodies indicating viral elimination and cure of the disease. The estimated incidence of this rare event in children is 0.05–0.8% per year in endemic areas with predominantly perinatal HBV transmission [7, 15].

Approximately 20–30% of inactive HBsAg carriers will experience spontaneous reactivation during long-term follow-up. Those episodes may cause progressive liver damage. The reactivation phase is characterized by the presence of anti-HBe and elevated aminotransferases. HBV DNA levels rise over 2000 IU/ml. This status is also named HBeAg-

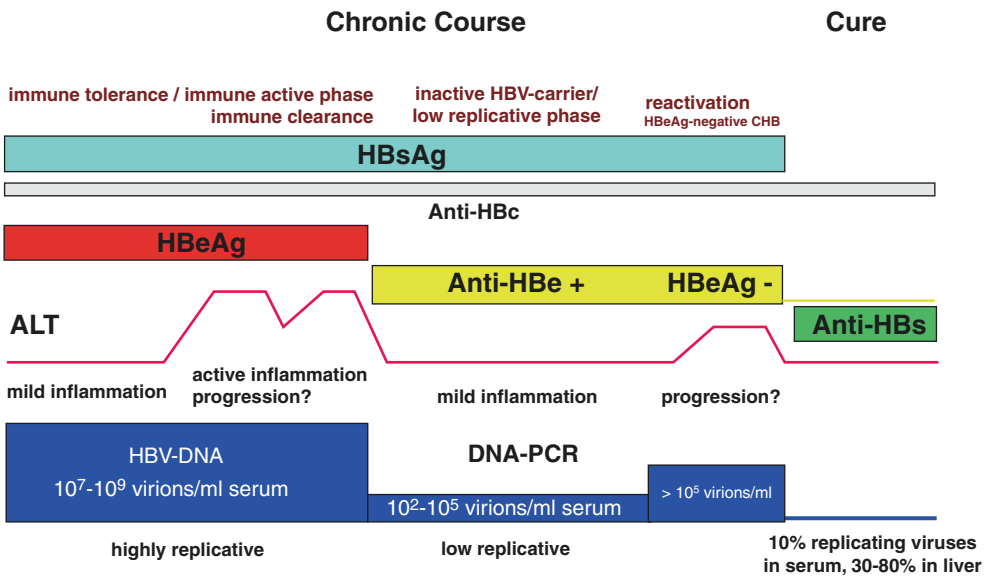

Fig. 63.1 Illustration of the different phases of chronic hepatitis B. *HBV-DNA* hepatitis B virus deoxyribonucleic acid, *DNA-PCR* deoxyribonucleic acid polymerase chain reaction, *HBV* hepatitis B virus, *HBsAg* hepatitis B surface antigen, *Anti-HBc* antibody against HBcAg, *ALT* alanine aminotransferase, *HBeAg* hepatitis e antigen, *Anti-HBs* antibody against HBsAg

negative hepatitis B. However, reactivation rarely occurs during childhood and adolescents.

Another particular condition warrants mention: occult HBV infection. It is defined as the existence of HBV DNA in serum among HBsAg-negative patients and can be classified into seropositive and seronegative with respect to the presence of anti-HBs or antibody against HBcAg (anti-HBc) antibodies. Possible explanations are low levels of viral replication activity or the emergence of HBV variants in the a-determinant of the S-gene. Occult hepatitis B is most common in endemic regions and seems rare with 1.4% [22]. However, prevalence may rise considerably in immunized children from HBsAg-positive mothers. One study reported a prevalence of 28% in this special group [23].

Long-Term Prognosis

Individuals with chronic HBV infection are at risk to develop long-term sequelae such as end-stage liver disease including liver cirrhosis, hepatic failure, and HCC. Progression strongly correlates with the disease activity in terms of viral replication level, inflammatory activity, HBsAg levels, HBV genotypes, and HBeAg/anti-HBe status. Strong risk factors for developing liver cirrhosis and HCC are higher age, male, presence of HBeAg, HBV DNA levels $>10^4$ copies/ml, HBsAg serum concentrations $>10^3$ IU/ml, and alanine aminotransferase (ALT) > 45 IU/l [24, 25]. Progression to liver cirrhosis in children is under 5% until adulthood, and data of the Asian region report 0.01–0.003% of individuals with chronic hepatitis B to be expected developing HCC in childhood [26, 27]. In general, anti-HBe seroconversion significantly reduces the risk of developing HCC. The time at which anti-HBe seroconversion occurs is important. A study in adults investigating the 15-year cumulative incidences of HBeAg-negative hepatitis demonstrated that cirrhosis and HCC increased with increasing age of HBeAg seroconversion [28]. The lowest risk was observed in patients with anti-HBe seroconversion under the age of 30 (cirrhosis 7%, HCC 2.1%) and highest in individuals older than 40 years (cirrhosis 42.9%, HCC 7.7%). The hazard ratio for HBeAg-negative hepatitis, cirrhosis, and HCC was 2.95, 17.6, and 5.22, respectively, in the older compared with the younger group. The authors concluded that patients with HBeAg seroconversion before age 30 have an excellent prognosis, whereas patients with delayed HBeAg seroconversion after age 40 have significantly higher incidences of HBeAg-negative hepatitis, cirrhosis, and HCC. An additional precondition is persistently normal ALT levels [29]. Since children have a high probability to experience anti-HBe seroconversion until adulthood, the overall risk of developing severe liver disease in later life seems limited. Nevertheless, there remain a considerable number of patients with immune tolerance or inflammatory activity that needs careful and professional monitoring.

Relevance of Genotypes and Mutants

During the replication cycle, HBV polymerase is acting as a reverse transcriptase without proofreading function. Therefore, mutant viral genomes are regularly emerging in a considerable number particularly during the high replicative status. Peculiar requirements such as replication modalities, selection pressure, and changing immunological conditions may select variants and strongly influence the predominant HBV quasispecies in an infected individual. Generally, a change of the primarily determined genotype is possible during long-term course and ranges between 2.8% and 19% usually associated with anti-HBe seroconversion [26, 30]. It is not yet known if there is any clinical impact at all. In adults, genotype C infection rather than genotype B is associated with a delayed anti-HBe seroconversion and a higher risk of developing HCC. Genotype D tends to proceed more severely and shows delayed anti-HBe seroconversion compared with genotype A. Precore and basic core promotor (BCP) mutants are frequently associated with HBeAg-negative hepatitis, and HBsAg escape mutants are now increasingly observed in association with primarily vaccinated children. The typical precore point mutant is the G1896A stop codon preventing the production of HBeAg. It emerges typically around the time of anti-HBe seroconversion and may be associated with a decreased risk of developing HCC compared with the wild type. But it can also be found in patients with HBeAg-negative hepatitis. Depending on European or Asian regions, precore mutants have been detected between 8% and 50% in HBeAg-negative children. The BCP mutants A1762T/G1764A prevail to be associated with an increased risk for HCC. But, finally, the data remain controversial [7, 16, 31, 32].

Treatment

Since there is no definite curative medical treatment available to date, it has to be defined what the aim of antiviral treatment should be in dependence on age group and phase of chronic hepatitis B. There is no doubt that one major goal is to reduce the risk of progressive liver disease and long-term sequelae such as liver cirrhosis, hepatic decompensation, and HCC and eventually to achieve the same life expectancy compared with healthy individuals of the same age. Unfortunately, anti-HBs seroconversion can only be reached in 5–10% at the most under current medical treatment strategies. Thus, the most important task in the treatment of children and adolescents is to achieve anti-HBe

seroconversion at the earliest possible time associated with suppressed viral replication and decreased liver inflammation followed by persistent presence of anti-HBe, undetectable HBV DNA, and preferably aminotransferase values less than half of the upper limit of normal. Children with HBeAg-positive hepatitis should be monitored every 6 months with physical examination, measurement of laboratory parameters such as aminotransferases, hepatitis B serology, alpha-fetoprotein, and ultrasound of the liver. After anti-HBe seroconversion, follow-up visits can be performed for lifetime on an annual basis [15, 17, 33].

The decision to treat should be based on age, phase of HBV infection determined by ALT level, HBeAg/anti-HBe status, liver histology, coexisting diseases, and expectable compliance. The response rate in patients in the immune-tolerant phase is very low. Thus, treatment of children with normal aminotransferases is not recommended. Recently published clinical trials in immune-tolerant children and adults combining a nucleoside analogue and peg-alpha interferon did not achieve a higher anti-HBe seroconversion rate compared to the control group [34, 35]. However, treatment should be considered when aminotransferases rise and transition to the immune-active phase is recognized. Children and adolescents who have persistently elevated ALT levels for more than 6 months should be offered treatment. Currently, seven treatment options are approved for hepatitis B in adults, including two formulations of conventional and pegylated interferon as an immunomodulatory therapy and five nucleos(t)ide analogues (lamivudine, adefovir dipivoxil, entecavir, telbivudine, and tenofovir disoproxil) with strong reduction of viral replication. Approval for children and adolescents depends on the region. Large trials have been performed in children for lamivudine, adefovir, entecavir, and tenofovir. Entecavir has been authorized from 2 years onward, and adefovir and tenofovir disoproxil have been approved for subjects older than 12 years of age in the USA and Europe [36–38]. Predictors of response may be increased ALT levels, relatively low HBV DNA levels, and infection with genotype A or B. The main problem of all clinical trials with nucleos(t)ide analogues is the duration of medical treatment of not more than 96 weeks. Although a high proportion of treated patients will experience a significant decrease of viral load, the anti-HBe seroconversion rate cannot be expected to exceed 25%. After ceasing treatment, a reactivation of viral replication to baseline levels can be observed. A 24-week course of alpha interferon yields an approximately 10% higher anti-HBe seroconversion rate. Anti-HBs seroconversion rate is limited to single cases with nucleos(t)ide analogues and may range between 6% and 10% in patients with alpha interferon. There is no doubt that these results are dissatisfying with respect to our primary goal of anti-HBe seroconversion. Another interesting fact is that alpha interferon treatment only accelerates anti-HBe seroconversion in successfully treated individuals but does not enhance the absolute number of responders [39]. Extending the treatment with nucleos(t)ide analogues for several years will result in an anti-HBe seroconversion rate of 40–50% [33]. However, there are no long-term data in children with regard to side effects. In the case of anti-HBe seroconversion, treatment should be maintained for 12 months, because the treatment-induced anti-HBe-positive status may be instable and reactivation may occur [40, 41].

In view of the present data and experience, there is a remarkable counseling conflict between the choice of drug and the duration of treatment, given that anti-HBe seroconversion remains the essential goal. Antiviral drug resistance is a major limitation to the long-term success of antiviral treatment. For this reason, lamivudine with a 5-year resistance rate of 70% has been considered obsolete just as adefovir which does often not sufficiently suppress viral replication. Nevertheless, at least for smaller children, lamivudine can be used as an approved drug for a limited time. Telbivudine has also a considerable resistance risk and is not approved. Entecavir and tenofovir do not show significant resistances after years of treatment. Tenofovir disoproxil may be associated with an increase in serum creatinine levels after 3–5 years of therapy. Decrease of bone mineral density has also been reported. These side effects might occur less frequent with the new tenofovir alafenamide. Oral treatment with nucleos(t)ide analogues is quite comfortable but needs a real true commitment to the treatment, and alpha interferon may have sometimes restrictive side effects but with the advantage of a defined duration.

Thus, the decision which treatment option to choose is not that easy and has to be achieved in agreement with the patient and the parents. Alpha interferon is particularly appropriate for those children and adolescents who are reluctant to commit to a long duration of treatment and are not in the pubertal growth spurt. Nowadays, peg-alpha interferon should be recommended for 48 weeks. Nucleos(t)ide analogues are most appropriate for patients with contraindications to interferon, after liver transplantation with an anti-HBc-positive donor or under immune suppressive treatment. It is most important that they are willing to commit to a treatment for several, probably 3–5, years, maybe longer. Entecavir and tenofovir have the best profile in terms of safety, efficacy, and drug resistance. For younger patients, entecavir seems actually the preferable option.

Children and adolescents with a HBeAg-negative hepatitis should be treated with a nucleos(t)ide analogue if ALT levels are elevated, and HBV DNA concentration is above 20,000 IU/ml to prevent progressive liver disease [42]. During long-term treatment with nucleos(t)ide analogues, HBV DNA, HBeAg/anti-HBe status, and aminotransferase levels should be monitored every 3 months. Very low or negative HBV DNA concentrations are important preconditions to avoid drug resistance.

Prevention

Vaccination is the most effective procedure in order to prevent infection with the HBV. Active and passive immunization is well established in newborns of HBsAg-positive mothers. The first injections have to be administered within 12–24 h after birth to achieve a seroprotective response in 90–95% when two monthly follow-up active vaccinations are completed. Very-low-birth-weight preterm infants should receive a total of four doses. HBeAg-positive mothers can be treated with a nucleoside analogue (telbivudine, tenofovir) during the last trimester of pregnancy to reduce the risk of vertical transmission [14].

In many countries, routine active HBV vaccination is implemented in the vaccination schedule of all infants. Postvaccination testing for a protective anti-HBs concentration (> 100 IU/l) is not routinely recommended. If indicated, the best time would be approximately 2–3 months after the last vaccination. Revaccination is indicated in subjects with an anti-HBs titer <10 IU/l. In the majority of nonresponders, three more vaccinations will induce protective response. According to present experiences, protective anti-HBs response will be maintained for more than 15 years [13, 41].

Chronic Hepatitis C

Introduction

Hepatitis C virus (HCV) infection is a frequent course of chronic liver disease, and approximately 71 million people are estimated to be chronically infected worldwide. The number of chronically infected children up to the age of 15 is estimated with approximately 13 million. Unfortunately, to date, no preventive vaccination could be developed. Despite a normally benign course of the disease during childhood and adolescence, there is a considerable lifetime risk of progressive liver disease, liver cirrhosis, and the development of a HCC, which may eventually reduce life expectancy. Remarkable advances have been made in therapeutic approaches so far, and considerable rates of cure have been yielded with the former interferon-based treatment standard of care. In the last years, interferon-free treatment regimen using direct-antiviral agents (DAA) has become the new standard of care, yielding excellent results for viral elimination. DAAs are now also available for children and adolescents.

Pathogenesis of Chronic Hepatitis C Infection

HCV is a positive-stranded RNA virus within the *Flaviviridiae* family. It forms its own genus *Hepacivirus*, and there are six main genotypes. The viral genome encodes nine proteins including its own RNA polymerase. Because of the high error rate of the virus-specific RNA polymerase, many variants may be produced. So-called quasispecies represent the high variability of the virus which allows a survival advantage to the virus. Replication of HCV starts with the binding to hepatocytes and entry which is a rather complex procedure. RNA is released into the cytoplasm and translated in the rough endoplasmatic reticulum. A 3000-amino-acid-long polypeptide arises and is then cleaved into ten different products. Membranous replication vesicles are induced, and HCV assembly is accomplished and released with the help of very-low-density lipoprotein (VLDL) synthesis. Chronic hepatitis C in children is associated with a variety of histological patterns, mostly considered as mild and slow progressive. Nevertheless, significant fibrosis or cirrhosis may occur but is not expected to exceed 4% until reaching adulthood. Need for liver transplantation is very rare as is the development of HCC [43]. Little information is available about the host response to the virus. Cluster of differentiation 4 (CD4) + lymphocytes seem to be involved. Infants with the rs 12,979,860 CC genotype for the IL28B polymorphism tend to experience a higher spontaneous viral elimination [44–46].

Epidemiology

The prevalence of HCV infection in children in developed countries ranges between 0.1% and 0.4%. For adults, prevalence rates are 0.4–3% in North America and Western Europe and higher in Eastern Europe and Middle East. Egypt has the highest prevalence with 9%, almost exclusively genotype 4 [47, 48]. Central and East Asia and North Africa are estimated to have a prevalence between 3.6% and 3.8% [49]. During the last 15 years, the predominant route of viral hepatitis C transmission has become vertical infection. Contamination through blood products is exceedingly rare in developed countries but may remain an issue in developing countries. The rate of perinatal transmission from an HCV-RNA-positive mother ranges from 2% to 5%. Out of this group, a considerable number of infants received the infection probably already in utero [50]. Concomitant HIV infection may increase the risk of HCV transmission. Breastfeeding does not promote viral transmission and is allowed. The HCV prevalence in pregnant women from North America and Central Europe was reported between 0.16% and 0.53%. Assumed a perinatal transmission rate of 2–4%, 8–10 newborns in 100,000 births per year may be infected and become chronically infected during the first year of life. Viral clearance in vertically infected children seems to be dependent on the genotype and was reported to range from 2.4% to 25%. In contrast, children infected with genotype 3 had a higher

spontaneous clearance rate compared to individuals with genotype 1. Beyond the age of 5 years, spontaneous viral elimination becomes less likely [51, 52]. In view of the fact that more and more patients can be cured with DAAs, it is expected that the prevalence of chronic hepatitis C infection will decrease significantly within the next 5 years.

Diagnosis

Serologic testing for anti-HCV antibodies is the appropriate screening test for HCV. The next diagnostic step is the determination of quantitative HCV RNA and the genotype. The most prevalent genotype in pediatric trials performed in Western countries was genotype 1 (ca. 74%) followed by genotype 3 (ca. 14%) and 2 (ca. 9%). Genotype 4 had the lowest prevalence (ca. 3%) [53]. It is useful to perform an ultrasound examination including liver stiffness assessment for the baseline report. As chronic hepatitis C usually is a histologically mild disease with low inflammatory activity in childhood, liver biopsy is not mandatory. However, in subjects, who are suspicious of progressive liver disease or cirrhosis or if there is an impact on therapeutic decisions, liver biopsy may be a reasonable measure [53].

Several studies have demonstrated that certain host polymorphisms (e.g., CC) located upstream of the IL28B (interferon lambda 3) gene are associated with a higher sustained viral response rate to combination treatment with peg-alpha interferon and ribavirin. There is also an association with spontaneous clearance of HCV. So far, the determination of IL28B polymorphisms has not been used routinely and is becoming far less important in the treatment with DAAs.

Natural History

Normally, HCV infection is asymptomatic. Histological findings are usually mild, and the risk of severe complications until the infected individuals are reaching adulthood is low. Not more than 5% of children and adolescents will have evidence of advanced liver fibrosis or cirrhosis. Liver transplantation units from the USA have been reported on 133 transplanted children due to chronic hepatitis C during a time span of 13 years. In a lifetime, the risk of developing liver cirrhosis is about 20%, and the risk of HCC based on liver cirrhosis is estimated to be 2–5% [45]. These data are from adults, and there are no long-term follow-up studies in vertically infected patients. Natural history is also affected by other medical and social factors. Overweight children with liver steatosis are at greater risk for progressive liver disease. Risky behaviors and alcohol misuse worsen the long-term prognosis. Similar course and progression of hepatitis C were reported in former pediatric patients with successfully treated malignant disease after three decades of observation with about 20% spontaneous clearance and up to 5% liver cirrhosis. During the chronic course, ALT levels may be normal or intermittently elevated. Only few patients show persistent markedly elevated aminotransferases. Also the HCV RNA serum concentration may considerably fluctuate but without immediate prognostic relevance. Spontaneous resolution beyond the preschool age is quite rare and may occur in up to 10% of adolescents [45, 48, 54]. Some extrahepatic manifestations may be associated with chronic hepatitis C such as glomerulonephritis and possibly cognitive deficits or developmental delay [55]. However, in adult patients, the clinical effects of reported symptoms such as fatigue, depression, or marginal poorer learning efficiency were rather limited [56]. In conclusion, early acquired chronic hepatitis C is a clinically and histologically silent and hidden condition. Nevertheless, it may become insidious. Although the rate of developing liver cirrhosis until adulthood is low, activation beyond the second decade of life is likely. A large Danish study in adults revealed that in patients with chronic HCV infection, the 8-year risk of liver-related death was 5.5% compared with 2.0% in individuals who cleared the infection [57]. However, patients who have eventually proceeded to compensated liver cirrhosis have a dubious prognosis. In a follow-up study of cirrhotic patients over more than 10 years, HCC developed in 32%, and the annual mortality rate was 4% [58]. Thus, the literal aim of therapeutic interventions in children and adolescents is not the treatment of an ongoing liver disease but the prevention of a future one by early eradication of the infection.

Treatment

The primary goal of HCV therapy is to cure the infection, which is reflected by persistently negative HCV RNA in serum and normalized aminotransferases. Sustained viral response (SVR) is defined as an undetectable HCV RNA level 24 weeks after cessation of treatment. The interferon-based treatment regimens included the combination of pegylated alpha interferon with ribavirin and a protease inhibitor. They were the approved and established standard of care in adults until the year 2014. With these regimens, considerable sustained viral response rates could be achieved ranging from more than 65% for genotype 1 to over 80% for genotype 2 and 3 patients [59]. Then, the interferon-free era started with the introduction of direct antiviral agents (DAA) focusing on genotypes 1 and 4 [60]. Genotypes 2 and 3 were also treated in different combinations over 12 to 24 weeks. The most important substance was sofosbuvir. However, more and more combinations were tested and approved, and numerous drugs were on the market only for a few years [61]. Table 63.1 summarizes the currently mostly used preparations with respect to the approval status in adults and children.

Table 63.1 Direct antiviral agents for the treatment of chronic hepatitis C

Substances	Registered trade name	Genotype	Trials in children	Approved >12 years of age [a]	Approved >3 years of age [a]
Sofosbuvir/Ledipasvir	Harvoni	1, 4	Yes	Yes	Yes
Ribavirin/Sofosbuvir [a]	Ribavirin, Sovaldi	2, 3	Yes	Yes	Yes
Ritonavir/Ombitasvir/Dasabuvir/ Ribavirin [a]	Viekirax, Exviera	1, 4	Yes	Not intended	Not intended
Daclatasvir/Sofosbuvir [b]	Daklinza, Sovaldi		Yes	Not intended	Not intended
Grazoprevir/Elbasvir	Zepatier	1, 4	Yes	In question	In question
Sofosbuvir/Velpatasvir	Epclusa	1–6	Yes	Yes	Yes, > 6 years
Sofosbuvir/Velpatasvir/Voxilaprevir [c]	Vosevi	1–6	Yes	Not intended	Not intended
Glecaprevir/Pibrentasvir [c]	Maviret	1–6	Yes	Yes	Yes

[a] Approval by FDA and EMA
[b] No longer in use in adults
[c] Duration of treatment 8 weeks in non-cirrhotic patients

Experiences with the treatment of children with chronic hepatitis C started in the early 1990s. Nineteen studies using recombinant alpha interferon were published between 1992 and 2003. A meta-analysis of trials with alpha interferon monotherapy showed a wide range of viral response (0–76%). Based on an increasing number of trials in adults, ribavirin was also added to alpha interferon treatment trials for children. Between the years 2000 and 2005, six studies were published showing a sustained viral response rate from 27% to 64% [52]. It became clear that genotype-2- and genotype-3-infected individuals responded much better. Alpha interferon-2b in combination with ribavirin was then approved by the FDA. Trials with peg-alpha interferon and ribavirin followed in the next years, and both peg-alpha interferon-2b and peg-alpha interferon-2a have been approved by FDA and EMA 2008/2009 and 2011/2012 in combination with ribavirin for children. Peg-alpha interferon and ribavirin therapies in treatment-naïve children and adolescents yield a sustained viral response rate in approximately 50% of adequately treated genotype-1-infected patients. Thus, this option could theoretically be offered to all interested individuals. In patients infected with genotype 2 or 3, treatment for 24 weeks has a response rate of more than 90% [62–64]. It could be used in children from 3 years and peg-alpha interferon-2a from 5 years onward, but it is also no longer recommended. Since spontaneous viral elimination in vertically infected subjects may occur within the first 3 to 4 years, the start of therapy was agreed after the age of three.

Treatment management of children with chronic hepatitis C infection is formed by the attitude of the medical attendant regarding the need of therapeutic intervention with respect to a generally slow progressive disease. In general, also for children and adolescents, interferon-based treatment options are considered obsolete, despite approval. Adverse events during treatment were frequent, and the duration was 24 to 48 weeks in dependence on the genotype. Under the aspect of health prevention for a long lifetime, all children with a measureable level of HCV RNA should be treated. The level neither of aminotransferases nor of HCV RNA predicts the long-term outcome of the disease. Also, liver histology is not a helpful entry criterion for indicating treatment, because children generally do not have severe lesions. With the development of the new DAAs, there is no reason not to treat children and adolescents after diagnosis of chronic hepatitis C from 3 years of age onward [65, 66]. The drugs are well tolerated and have only an exceptionally low rate of side effects.

Three groups of DAAs were differentiated in three groups: NS3/4A protease inhibitors, NS5a inhibitors, and polymerase inhibitors. Protease inhibitors have the ending "previr," NS5a inhibitors have the ending "asvir," and polymerase inhibitors are ending with "buvir." Sofosbuvir is one of the most potent polymerase inhibitors. It is important to combine at least two of different groups to avoid developing rapid drug resistance.

For the pediatric population, several clinical trials have been performed. Due to the approval regulations, the first groups included were between 13 and 18 years of age, followed by the younger ages. The combination of sofosbuvir and ledipasvir is approved from 3 years onward and suitable for the treatment of genotypes 1 and 4. It is administered for 12 weeks [67–69]. For genotypes 2 and 3, sofosbuvir and ribavirin are approved. Genotype 2 has to be treated for 12 weeks and genotype 3 for 24 weeks [70, 71], which can now be considered second line. The most recent approval is the combination of glecaprevir and pibrentasvir, which is pangenotypic (genotypes 1–6) and has the additional advantage of an 8-week treatment duration [72]. With Sofobuvir/Velpatasvir there are now two pangenotypic active drugs available. This panel seems sufficient to successfully addressing the pediatric chronic HCV infections. The overall sustained viral response rate was extremely high and achieved in most of the trials 100%. That was also true for individuals who were treatment experienced with a previous interferon-based therapy. Relapses were observed only in few isolated cases. It is recommended to determine HCV RNA 4 weeks after the start of treatment. Most patients are already negative at that time. The most important checkpoint is SVR

24, 24 weeks after cessation of treatment, as HCV RNA negativity is associated with long-term elimination. The safety and tolerability profile of the DAAs is also excellent. The vast majority of adverse events were mild and unrelated to the medication. Headache, fatigue, or signs of upper respiratory tract infections were registered up to about 15% [72]. In adult trials, there was no difference to the side effects of the placebo arm. Treatment does not appear to have an impact on growth and development.

Overall, early treatment of the pediatric population with chronic hepatitis C will decrease the pool of infected individuals and not only alleviate the development of a progressive liver disease but also prevent further transmission in the hope to eradicate the disease in this age group.

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Bacterial, Fungal and Parasitic Infections of the Liver

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Anita Verma

Introduction

Primary nonviral infections of the liver parenchyma itself are uncommon; presumably the phagocytic Kupffer cells play a key role in preventing the infection. The liver's dual blood supply renders it uniquely susceptible to infection, receives blood from the intestinal tract via the hepatic portal system and is sustained by systemic circulation via the hepatic artery. Because of this unique perfusion, the liver is frequently exposed to systemic or intestinal infections or the mediators of toxaemia. The biliary tree provides a further conduit for gut bacteria or parasites to access the liver parenchyma.

Infections of the liver with a wide range of organisms present variously from asymptomatic biochemical abnormalities to symptomatic hepatitis or space-occupying lesions, for example, abscesses or granulomata producing biochemical changes of cholestasis but rarely significant jaundice. Some of these infections have a high mortality if not treated promptly. We describe nonviral infectious diseases affecting the liver caused by bacteria, mycobacteria, spirochaetes, rickettsia, fungi and parasites.

Bacterial, Spirochaetal and Rickettsial Infections

Bacterial Sepsis

Bacterial sepsis precipitating jaundice is a well-recognized phenomenon particularly in the newborn and young infants [1]. The exact pathogenesis of hepatic insult is not known but may be multifactorial, including direct invasion of liver parenchyma by blood-borne pathogens and nonspecific injury due to hypoxia or endotoxin-mediated paralysis of biliary canaliculi inducing cholestasis. Implicated bacteria include 'coliforms', pseudomonads, *Salmonella* spp., anaerobes, *Haemophilus influenzae*, streptococci and *Staphylococcus aureus.* In patients with jaundice, serum bilirubin is usually between 5 and 10 mg/dl. Hepatomegaly is found in 50% of cases, and liver enzymes are usually mildly elevated. Clinical evaluation and microbiological investigation may identify the source of sepsis, and antimicrobial therapy usually results in complete resolution.

Liver Abscess

Pyogenic liver abscess (PLA) in infancy and childhood is uncommon, with incidence ranging from 25 to 400 per 100,000, but carries a high mortality [2–4]. PLA is uncommon in developed countries, but higher prevalence has been reported from developing countries [2–5]. Though PLA in healthy children is a rare entity, up to 40–50% has occurred among immunocompromised children [2]. Pyogenic bacteria can reach the liver through various routes: (i) portal, secondary to gut pathologies such as appendicitis, inflammatory bowel disease or diverticulitis, sometimes complicated by portal pyelophlebitis and portal vein system thrombosis; (ii) biliary, caused by extrahepatic biliary tract disease such as stricture, calculus or malignancy; (iii) blood-borne from an infected focus anywhere in the body via hepatic artery; (iv) contiguous extension from gallbladder or perinephric abscess; (v) following penetrating wounds of liver; and (vi) cryptogenic [2, 5].

PLA may present as single large lesion or multiple abscesses, the latter are often secondary to biliary tract infection. The importance of the portal venous route has fallen with better diagnosis and management of appendicitis. In most reviews, more than 60% abscesses are in the right lobe, 20–25% bilateral and less than 15% in the left lobe [4, 5]. Predisposing factors include immunosuppression, quantitative or qualitative granulocyte abnormalities like

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chronic granulomatous disease, trauma, umbilical vein catheterization, omphalitis, sickle-cell disease, biliary tract surgery, hepatic artery thrombosis (post-liver transplantation), liver biopsy, percutaneous or endoscopic biliary drainage, diabetes, worm infestation and protein-energy malnutrition especially in developing countries [4, 5].

Multiple abscesses complicate biliary diseases such as bacterial cholangitis, sclerosing cholangitis, congenital biliary anomalies (Caroli's disease) and gallstones with higher mortality. The aetiology of PLA is variable. *S. aureus* is the most common isolate reported in children from developed world and mostly associated with underlying condition of chronic granulomatous disease [5]. Gram-negative aerobes (*Klebsiella* spp., *E. coli*) were the most common organisms reported from developing countries [2, 4, 5]. Less frequent causes include, e.g. anaerobes, microaerophilic streptococci, *Pseudomonas* spp., *Clostridium* spp., *Salmonella typhi, Yersinia enterocolitica* and *Pasteurella multocida*.

The classic presentation is pyrexia, chills, right upper quadrant (RUQ) tenderness, abdominal pain, hepatomegaly and leucocytosis but may be nonspecific. The diagnosis must be entertained in any pyrexia of unknown origin (PUO). Unusual presentations include an abdominal mass or acute abdomen secondary to rupture into the peritoneal cavity or portal hypertension secondary to portal pyaemia and portal vein thrombosis. Liver function tests may be unhelpful with nonspecific changes. Ultrasonography (USS), computerized tomography (CT) and magnetic resonance imaging (MRI) are all sensitive but cannot always differentiate abscesses from other lesions such as cysts, tumours or haemorrhage. USS- or CT-guided drainage of as much pus as possible (from as many abscesses as possible) confirms diagnosis and is central to the management. Initial treatment is conservative with broad-spectrum antibiotics (e.g. cefotaxime plus metronidazole or clindamycin) and should be adjusted when culture results are available [5, 6]. Duration of treatment is usually 3–6 weeks. Patients with multiple abscesses have to be on conservative treatment after a diagnostic tap, and up to 3–4 months of antibiotic therapy has been recommended to prevent relapses [5]. Prognosis is worse in multiple abscesses. Most reports emphasize the good outcome after percutaneous drainage, which should be USS or CT guided [6, 7]. Contraindications to drainage include ascites and inaccessible lesions. Complications of aspiration include haemorrhage, hepatic laceration, fistula formation, peritonitis and additional abscess formation. Indications for open drainage procedure are biliary obstruction, loculated or highly viscous abscesses, persistence of fever for more than 2 weeks despite percutaneous catheter drainage and appropriate antimicrobial therapy [7]. Predisposing immunodeficiency conditions should be managed with appropriate expert opinion from immunologists or infectious disease experts.

Cholangitis

The normal biliary tract is sterile, and, in children, acute cholangitis rarely occurs in the absence of congenital abnormalities or interventions to the biliary tract [8]. The children at highest risk include those with portoenterostomy or choledochal cyst, those with underlying condition of primary sclerosing cholangitis and those who have nonoperative biliary manipulations such as transhepatic cholangiography or endoscopic retrograde cholangiography with stent placement. Risk of cholangitis in children after Kasai operation has been reported to be 40–50% [8]. Partial biliary obstruction encourages bacterial growth, with increased intraductal pressure and reflux of bacteria into blood vessels and perihepatic lymphatics leading to bacteraemia. Infection may ascend from the duodenum or an infected gallbladder or via lymphatics or bloodstream [9].

Cholangitis is a clinical diagnosis based on fever, abdominal pain, jaundice, pale stools or hepatic tenderness. However, the spectrum encompasses mild disease to severe sepsis, or shock, with bacteraemia [8]. Although *Escherichia coli, Klebsiella* spp., *Enterobacter* spp. and *Pseudomonas* spp. are usually implicated, infection may be polymicrobial and include anaerobes [8]. Leucocytosis is common, but changes in liver function tests are nonspecific; the serum bilirubin may be normal. In recurrent cholangitis, liver biopsy may be indicated for confirmation and microbiological examination. Treatment requires supportive care, and an urgent USS or CT will help establish whether obstruction requires drainage. Broad-spectrum antibiotics should be administered—such as an acylureidopenicillin (piperacillin, mezlocillin or piperacillin-tazobactam) or a late-generation cephalosporin (e.g. ceftazidime), plus an aminoglycoside [10]. Single agents, such as piperacillin-tazobactam, ciprofloxacin and imipenem or meropenem, appear safe and effective if an aminoglycoside is contraindicated. Duration of treatment is generally 3 weeks for acute cholangitis, but prolonged therapy may be necessary for recurrent cholangitis and multiple resistant bacteria [11]. Three months of intravenous antibiotics through central line has been helpful in treating recurrent cholangitis in biliary atresia children with portoenterostomy. Prolonged antimicrobial therapy in recurrent cholangitis only helps in preventing the bacteraemia by preventing bacterial overgrowth in biliary tract. With prolonged antibiotic therapy, there is always risk of development of resistant organisms and therefore more serious infection.

Tuberculosis

Tuberculosis (TB) of the liver is almost invariably a complication of miliary disease and occurs in 50% and 75% of patients with pulmonary or extrapulmonary TB, respectively.

The site of primary focus usually dictates presentation. Rarely the liver appears to be the sole site of infection such as in congenital TB acquired via the ductus venosus [12, 13]. Congenital TB may present in first few weeks of life with failure to thrive, hepatosplenomegaly and jaundice. In older children, hepatic TB presents with PUO, weight loss, abdominal discomfort and hepatomegaly [12, 13]. In areas of low incidence, a positive tuberculin skin test and quantiferon blood test (interferon-gamma release assay) is diagnostically useful. However, confirmation requires liver biopsy, histology and culture confirmation. Caseating granulomata on liver biopsy are highly suggestive of TB but may be absent; a granulomatous hepatitis can complicate bacillus Calmette–Guerin (BCG) administration. The diagnosis of TB should be sought by specific staining and culture of material from other sites, including bronchoalveolar lavage, lymph node or pleural biopsy, marrow aspirate, lumbar puncture or early morning gastric aspirates, as clinically indicated. Polymerase chain reaction (PCR)-based tests may be helpful but require further evaluation. Standard antituberculous therapy is effective, but expert advice should be sought in areas with a high incidence of drug-resistant TB or in compromised patients, including those with concurrent HIV infection.

Brucellosis

It is a multisystem infection caused by *Brucella melitensis, B. suis, B. abortus* and *B. canis; B. melitensis* causes more severe disease with a higher risk of chronicity. In endemic areas, transmission is often by ingestion of unpasteurized dairy products or raw meat. Granulomatous hepatitis may occur in acute or chronic disease and manifests as nonspecific changes in liver function tests [14]. Diagnosis requires clinical suspicion, blood and bone marrow cultures, serology and histopathological examination. PCR-based tests for brucellosis are available. The recommended treatment for children under 9 years of age with uncomplicated brucellosis is trimethoprim–sulphamethoxazole. For the treatment of serious infection, the addition of gentamicin or streptomycin is recommended for the first 1–2 weeks. Older children should receive doxycycline (6 weeks) plus rifampicin or streptomycin (2 weeks).

Listeriosis

Listeria monocytogenes may cause liver disease as part of systemic intrauterine infection of the foetus—granulomatosis infantiseptica at birth or later in the neonatal period—and in older immunocompromised children after ingestion of contaminated food or water. The major hepatic manifestation is granuloma; jaundice is rare. Diagnosis is achieved by recovering the bacterium from blood culture, cerebrospinal fluid or liver aspirates. Treatment is with high-dose ampicillin, with or without gentamicin.

Tularemia

Francisella tularensis has been isolated from many wild mammals, domestic animals and birds. Human infection usually follows bites from parasites of these animals or direct contact with animals. In some cases, a hepatitis-like picture follows with raised aminotransferases. Hepatomegaly is rare and biopsy may show necrosis. Diagnosis is usually serological as the bacterium is difficult to recover in culture. Treatment with streptomycin or gentamicin is effective; the fluoroquinolones appear promising but require further evaluation.

Leptospirosis

Human infection follows exposure to leptospires excreted in the urine of chronically infected animals—including rats, cattle and dogs—or water contaminated with urine. Children are usually infected when swimming in contaminated rivers, ponds or lakes or by canine exposure [15]. Leptospires gain entry via skin abrasions, conjunctivae or mucosae, and an initial leptospiremia presages a multisystem infection. The incubation period is 5–15 days, and in 90% of patients there is a self-limiting anicteric disease, but 5–10% develop jaundice (Weil's disease) [16]. Classically, the anicteric leptospirosis runs a biphasic course, with a leptospiremic phase lasting for 3–7 days and a second phase associated with leptospiruria and rising antibody titres lasting for 4–30 days. Predominant manifestations are fever, headache, myalgia, abdominal pain, nausea, vomiting, meningism, conjunctival suffusion, maculopapular rash, impaired renal function, lymphadenopathy and hepatosplenomegaly. Weil's disease is characterized by hepatic, renal and vascular dysfunction with persistent fever, profound jaundice, abdominal pain, renal failure, confusion, epistaxis, haematuria, gastrointestinal bleeding and other haemorrhagic phenomenon. Death may follow cardiovascular collapse, renal failure and gastrointestinal or pulmonary haemorrhage, though with supportive therapy mortality should be less than 10%. Liver histology reveals swollen perivenular hepatocytes with increased mitoses indicative of regeneration and disorganized liver cell plates. Leptospires may be recovered from blood, urine or cerebrospinal fluid (CSF) during the first week of illness, and from urine thereafter, and may be seen by dark ground microscopy of blood in the early stage of disease and in urine thereafter. Diagnosis, however, is usually serological using complement fixation tests (CFTs), enzyme-linked immunosorbent assay (ELISA)

or a microagglutination test. PCR can detect leptospiral DNA in blood, serum, CSF, urine or aqueous humour. Penicillin or doxycycline is recommended and most beneficial if started early in the disease: The benefits of commencing antimicrobials later in Weil's disease are less clear.

Borreliosis

Borrelia burgdorferi is a tick-borne spirochaete which causes systemic infection in humans (Lyme disease) following exposure to these vectors in forest or parkland. Predominant manifestations of acute disease are fever, malaise, extending erythematous rash, meningism, arthralgia, hepatitis and lymphadenopathy. Abnormal liver function tests occur in up to 20% of patients and, rarely, hepatomegaly and RUQ tenderness [17]. Diagnosis requires clinical suspicion, positive serology and histopathology. Spirochaetes may be seen in liver biopsy with a mixed inflammatory infiltrates in sinusoids, mitotic activity and ballooning degeneration of hepatocytes with hyperplastic Kupffer cells. Ampicillin or amoxicillin is administered for 3 weeks in early disease in children less than 9 years of age and tetracycline for older children. In late disease, intravenous cefotaxime or ceftriaxone for 2–4 weeks is recommended followed by oral ampicillin plus probenecid for a further 4–8 weeks.

Syphilis

Treponema pallidum may infect the foetus at any stage of maternal syphilis, causing disseminated infection. Congenital syphilis may result in mucocutaneous lesions, a diffuse rash, pneumonitis, myocarditis, hepatosplenomegaly, jaundice, lymphadenopathy, haemolytic anaemia, thrombocytopoenia, perichondritis and osteochondritis; the infant is usually small for age. Late stigmata include arthropathy with bilateral knee effusions, notched upper incisors and frontal bossing of the skull and poorly developed maxillae. Neonatal death usually results from liver failure, severe pneumonia or pulmonary haemorrhage. Diagnosis requires detection of spirochaetes by darkfield examination (skin rash, nasal secretions) or serology, including detection of specific IgM antibodies; long bone radiography at 1–3 months of age may contribute to diagnosis. Benzylpenicillin (10–14 days) remains the drug of choice.

Q Fever

Q fever is a systemic infection caused by the rickettsia *Coxiella burnetii* following exposure to infectious dust or aerosols from farm or domestic animals or consumption of raw milk. Illness is usually a self-limiting 'flu-like illness' with an incubation period of 1–2 weeks. However, acute Q fever can present with atypical pneumonia and hepatitis with jaundice, hepatomegaly and abnormal liver function tests [18]. The characteristic histopathological finding is granulomata with dense fibrin rings around central lipid vacuoles. Diagnosis is by detecting immunoglobulin G (IgG) and immunoglobulin M (IgM) to phase II antigens of *C. burnetii*, usually by indirect fluorescent antibody test or ELISA. Seroconversion occurs at 7–15 days after the onset of symptoms with 90% patients having detectable antibodies by the third week. Titres of antibodies to phase I antigens exceed those to phase II antigens in chronic disease. Treatment is recommended for all cases to prevent chronicity; doxycycline or chloramphenicol is effective. Treatment duration depends on if it is acute or chronic Q fever. For acute infection, it is 2–3 weeks. But in case of chronic infection it can be as long as 12–18 months.

Parasitic Infections

Amoebiasis

Entamoeba histolytica is most commonly encountered in the tropics and subtropics. Hepatic abscess is a major complication of invasive amoebiasis and seen in 3–9% of adult cases but is less common in children [19]. Amoebic trophozoites reach the liver via the portal vein and induce hepatocyte apoptosis and a leucocyte response resulting in abscesses containing viscous brown pus. Hepatic abscesses can be demonstrated by USS or CT scanning and are usually single and most frequent in the right lobe. Multiple abscesses may be associated with more severe disease. A typical presentation is with pyrexia (75%) and RUQ pain radiating to the right shoulder. In left lobe disease, there may be epigastric or left shoulder pain. Tenderness in the hypochondrium (85%), tender hepatomegaly (80%) and localized swelling over the liver (10%) may be elicited [20]. Less specific symptoms include nausea, vomiting, concurrent diarrhoea or dysentery (10%) and loss of weight. Jaundice is present in up to 8% of cases. The white blood cell count is usually elevated. Demonstrating cysts in stool may contribute to diagnosis, but serum antibodies are present in more than 95% of patients. Aspiration under USS guidance may yield 'anchovy sauce' pus; rarely amoebas are seen in necrotic abscess wall or adjacent parenchyma. Abscesses may rupture into the peritoneal cavity, pleural cavity or lungs, pericardium, portal vein or biliary tract, intraperitoneal rupture being more common than intrathoracic. Extraintestinal amoebiasis should be treated with metronidazole or dehydroemetine for at least 2 weeks [21]. The cure rate with both the drugs are same, but metronidazole has the advantage of being less toxic and being effective for both hepatic and intestinal phases of the

disease. To prevent continued intraluminal infection, luminal amoebicide, such as paromomycin or diloxanide furoate, should be given. Occasionally, amoebic abscesses do not respond to metronidazole, and addition of daily chloroquine may be considered for 2–3 weeks. Chloroquine has an additive effect to metronidazole and better penetration of the abscess wall. Percutaneous needle aspiration is recommended for large abscesses, failure to respond or if there is imminent risk of rupture, particularly in to the pericardium.

Schistosomiasis

Schistosomiasis affects 200 million people worldwide, the majority of children aged 5–15 years [22]. Transmission occurs in endemic areas (Middle East, Brazil, West Indies, Far East and Southeast Asia) after exposure to water inhabited by infected snails. The intermediate hosts larvae (cercariae) released from snails can penetrate intact skin and disseminate. *S. japonicum, S. mansoni* and *S. mekongi* cause hepatosplenic disease subsequent upon portal venous system obstruction by a granulomatous response to eggs and subsequent periportal fibrosis and portal hypertension. Granulomata consist of eosinophils, epithelioid cells, plasma cells and lymphocytes encircling an ovum. Patients present with pyrexia, urticaria, eosinophilia, hepatosplenomegaly or upper gastrointestinal tract bleeding from oesophageal varices. Dilated abdominal wall veins and ascites reflect portal venous hypertension, and USS may reveal thickening of the portal vein. Ova should be sought in stool and urine and may be identified in liver or rectal mucosal biopsy. Serological tests cannot distinguish past from active infection, but a negative ELISA excludes the diagnosis. Praziquantel is the drug of choice; oxamniquine an alternative for *S. mansoni*.

Hydatid Disease

Echinococcus granulosus is the dog tapeworm. Typically, dogs are infected by being fed offal from infected livestock—such as sheep—which contain hydatid cysts. Humans become infected by close exposure to domestic dogs and the eggs passed in their faeces. The liver is most frequent site for cyst formation, usually in the right lobe (60–80%). Presentation is with hepatic enlargement, with or without palpable mass, epigastric pain, nausea and vomiting; secondary cyst pressure effects include portal hypertension, inferior vena cava compression or thrombosis and biliary cirrhosis. USS of the liver reveals round solitary or multiple cysts of variable size with multiple internal daughter cysts; calcification may be noted. Diagnosis requires demonstration of specific antibody by ELISA, CFT, indirect agglutination or latex agglutination tests. Closed aspiration should not be undertaken: definitive therapy requires surgical removal which may be complicated by spillage of contents, anaphylaxis and seeding of new cysts. To avoid this, cysts can be injected with chlorhexidine or hydrogen peroxide prior to surgery. Both mebendazole and albendazole may successfully treat small uncomplicated cysts.

Ascariasis

Adult *Ascaris lumbricoides* worms cause disease by migrating into the pancreatic ducts, gallbladder and biliary tract. Biliary ascariasis is more common in children than in adults. Children with a heavy worm burden may be malnourished. Presentation of biliary involvement includes fever, RUQ pain, vomiting and passing of worms in stool or vomitus. Mechanical obstruction by worms can cause acute cholecystitis, cholangitis and biliary colic. Adult worms in the biliary tract may be demonstrated by USS, cholangiography or endoscopic retrograde cholangiography (ERCP); eggs should be sought in the faeces [23]. Treatment is usually with mebendazole or albendazole—though endoscopic removal of adult worms may be necessary if there are persisting biliary symptoms.

Toxocariasis

Toxocariasis is caused by infection with the dog roundworm *Toxocara canis.* Ingested eggs hatch in the small intestine, and larvae penetrate the mucosa before migrating to the liver, lungs and many other tissues. Visceral larva migrans (VLM) refers to a syndrome of eosinophilia, pyrexia, leucocytosis, hepatosplenomegaly, lymphadenopathy and hyperglobulinemia as the larva migrates. Ocular disease may also occur. A history of exposure to puppies should be sought. Humans are a 'dead-end' host: adult worms do not develop so eggs are not passed. Serodiagnosis is with an ELISA. In massive infection, liver biopsy may reveal eosinophils surrounding larvae or granulomata formation with epithelioid giant cells and lymphocytes. VLM is treated with thiabendazole. Severe disseminated disease or occular infection may warrant concomitant steroids.

Liver Fluke Infestation

Fasciola hepatica infection follows ingestion of aquatic plants—such as watercress—contaminated with eggs passed by infected sheep or cattle. After hatching, larvae penetrate the gut wall, enter the peritoneum and having breached the liver capsule pass through the parenchyma to the bile ducts. The flukes mature and lay eggs in the biliary tract, causing cholangitis and hepatomegaly. Diagnosis is made by demon-

strating ova in stool and positive serology. ERCP may show filling defects due to inflammation, and worms can be aspirated. Liver biopsy shows infiltration with eosinophils, histiocytes and polymorphs; granulomas may or may not be present. Treatment is with bithionol.

Clonorchis sinensis is endemic in the Far East. Infection follows ingestion of cysts in uncooked freshwater fish or crabs. Metacercariae excyst in the small intestine and invade the bile ducts in which the flukes mature, producing eggs which may be demonstrated in the faeces. Infection is usually asymptomatic, but bile duct fibrosis with liver impairment, strictures and pancreatitis may ensue. Praziquantel is the treatment of choice.

Toxoplasmosis

In the immunocompetent patient, acute acquired *Toxoplasma gondii* infection usually presents as self-limiting lymphadenopathy, though hepatosplenomegaly and hepatitis can occur. The risk and severity of congenital toxoplasmosis vary according to the trimester in which maternal infection with the protozoan occurs. The likelihood of foetal infection increases through pregnancy, whilst disease severity decreases. The spectrum of disease in infected neonates includes retinochoroiditis, meningoencephalitis, hydrocephalus, intracranial calcification, pneumonitis, myocarditis, purpura, hepatitis, hepatosplenomegaly and hydrops fetalis. Congenital toxoplasmosis must be differentiated from the other major causes of congenital infection: rubella virus, cytomegalovirus, herpes simplex virus, *T. pallidum* and *L. monocytogenes*.

Diagnosis of congenital infection requires full clinical evaluation and exclusion of other infections, with recovery of *T. gondii*, histology or serological investigation. Reference laboratory tests include culture (blood, body fluids, placenta), the dye test (for serum and CSF) and ELISAs for IgG, IgM, IgA and IgE detection in both neonate and mother. *T. gondii* DNA may be detected in body fluids (blood, urine and CSF) by PCR.

Treatment is with pyrimethamine plus sulphadiazine, though expert advice should be sought. Toxoplasmosis in the immunocompromised may follow reactivation or new acquisition and usually presents as central nervous system disease though other organs, including the liver, may be affected. *T. gondii* may be transmitted in the graft at liver transplantation and cause liver and severe systemic disease thereafter.

Fungal Infections

Fungal infections of the liver are usually seen in the immunocompromised—including those with acute liver failure. Although *Candida albicans* predominates, other *Candida* spp. and *Aspergillus* spp. infections are increasingly reported.

Hepatic Candidiasis

Severely immunocompromised patients are prone to disseminated candida infections with the liver (and often spleen) affected in 50–70%. Typically, hepatosplenic candidiasis presents in haemato-oncology patients rendered neutropenic at the time of recovery of the neutrophil count. The other risk factor for hepatic candidiasis is hepatic artery thrombosis in liver transplant patients. The hallmark is multiple small lesions in the liver and spleen on USS or CT scan with raised alkaline phosphatase. Yeasts or pseudohyphae may be visible on fine needle aspiration or in liver biopsy, and cultures may be positive; blood cultures are usually negative. Serology test such as detection of circulating cell wall antigen beta-D-glucan by ELISA and PCR-based tests can be used an adjunct for diagnosis of hepatic candidiasis. Hepatic or hepatosplenic candidiasis is treated with amphotericin B or micafungin or fluconazole. Although prolonged therapy may be required, the immune status of the patient is the key determinant of outcome.

Aspergillosis

Disseminated aspergillosis is an increasingly recognized problem in the severely immunocompromised. Increased incidence is seen in hospital with building constructions. Hepatic aspergillosis may manifest as an aspergilloma or granulomata formation, with hepatomegaly, elevated bilirubin, alkaline phosphatase and aminotransferases. Confident diagnosis requires both histopathological demonstration of invading hyphae and isolation of *Aspergillus* spp. (usually *A. fumigatus*) from liver biopsy. To date, serology has been unhelpful, but detection of circulating cell wall galactomannan by ELISA- and PCR-based tests shows promise. Treatment with voriconazole and liposomal amphotericin B should be commenced immediately—on clinical suspicion alone—in immunocompromised patients.

Other Rare Fungal Infections

Other rare fungal infections of the liver include histoplasmosis, blastomycosis, coccidioidomycosis and paracoccidioidomycosis.

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Liver Disease in Primary Immunodeficiencies

65

Nedim Hadzic

Introduction

Primary immunodeficiencies (PIDs) are heritable disorders of innate and/or acquired immunity, often complicated by severe, recurrent or unusual infections, chronic diarrhoea with failure to thrive and malignancies of lymphoid tissue in long-term survivors [1, 2].

Many of the problems children with these conditions face have been successfully addressed; for the early-presenting, life-threatening PIDs such as variants of severe combined immune deficiency (SCID) or hyper-immunoglobulin E (IgE) syndrome, elective hematopoietic stem cell transplantation (HSCT), preferably from haploidentical donors, has become a treatment of choice [3]. In contrast, many milder forms of PIDs can be successfully managed for short-to-medium term by regular anti-infectious prophylaxis with antibiotics and/or antifungals and replacement immunoglobulins. Next-generation sequencing has dramatically improved the diagnostic pathways by development of targeted molecular genetics panel but also introduced additional queries about relevance of heterozygous states and variants of unknown significance [4].

Liver disease occurs in approximately 25% of children with PIDs [5]. The proportion of adult patients developing this complication is unknown but likely to be underdiagnosed and overall higher. Their number and liver pathology potentially could be affected by exposure to alcohol and propensity to develop diabetes mellitus and obesity-related liver problems such as nonalcoholic fatty liver disease later in life.

Pathophysiology

Many of the liver-related problems in PIDs stem from the fact that compromised first-line anti-infectious barrier in gastrointestinal tract allows low-grade chronic infection which could ascend proximally into the biliary system and the liver. The infections are frequently caused by unusual pathogens, escaping routine anti-infectious strategies, such as *Cryptosporidium* (CS) or *Microsporidium* [5]. The main form of liver disease in PIDs is chronic cholangiopathy, seen in around 65% of affected children [5]. This condition has a well-documented potential to develop biliary cirrhosis and full-blown clinical features of end-stage chronic liver disease, such as portal hypertension, bleeding from oesophageal varices and hypoalbuminaemia with jaundice. In a small proportion of PID patients with the liver involvement, milder and more nonspecific histological features, such as fatty change, mild portal inflammation or development of small non-caseating intrahepatic granulomas, could be observed [5].

A considerable role in the chronic cholangiopathy of PIDs is attributed to protozoal infections, unaffected by the standard anti-infectious regimens. Chronic cryptosporidial infection has been implicated in chronic inflammatory and dysplastic biliary changes in the context of chronic cholangiopathy and superimposed complications such as biliary cirrhosis and cholangiocarcinoma. The pathogenic role of CS in immune deficiency is likely, albeit incompletely understood. Animal models of the CS-related cholangiopathy have been described. For example, interferon-gamma knockout mice appear to be particularly susceptible to CS infection, suggesting important role for this cytokine in pathogenesis of the cholangiopathy in PIDs [6]. The biliary injury in humans appears to be caused by direct cytopathic effects of this protozoan, mediated by apoptosis [7]. In addition, CS is known to cause cholangiopathy in human immunodeficiency virus (HIV) infection [8] and after organ transplantation [9]. Several additional intracellular parasites such as

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Microsporidium, *Mycobacterium avium intracellulare* and cytomegalovirus (CMV) have also been implicated in development of the chronic cholangiopathies in adult HIV-positive patients [8]. The accelerated evolution of the cholangiopathy in this setting is often interpreted by probable synergistic effects of multiple biliary infections [8].

A classical paradigm for development of the cholangiopathy of PIDs is hyper-IgM syndrome. This condition is caused by mutations in cluster of differentiation (CD)40 ligand (CD154) gene, known to control interaction between CD40 molecules from B-cells and CD40 ligand on activated lymphocytes in its X-linked form (CD40 ligand deficiency). Alternative mechanism for hyper-IgM syndrome is defective expression of immune activation-induced cytidine deaminase (AID) on B cells in the autosomal recessive form of the disease [10]. As a consequence, in both forms, B cells could not facilitate physiologic IgM class switching to other immunoglobulin types, which renders total serum IgM levels high and distal components of the immune response arm ineffective. However, the IgM levels may not always be elevated, and chronic neutropenia is often observed [11, 12]. Several series have described development of chronic biliary disease occasionally complicated by cholangiocarcinoma in older patients with hyper-igM syndrome. Another condition, recently described to predispose patients to develop chronic cholangiopathy, is dedicator of cytokinesis (DOCK)-8 deficiency, previously known as hyper-IgE syndrome [13].

Although there are hundreds of described PIDs, a majority now characterised by mutations in different genes encoding various molecular mediators of the immune response, frequent lack of genotype/phenotype correlation makes general recommendations for detecting liver disease difficult. On occasion, an adult patient could be diagnosed with PIDs, and exceptionally some children with conditions known to confer susceptibility for chronic cholangiopathy, such as hyper-IgM syndrome, could remain completely asymptomatic, on anti-infectious prophylaxis, well into the adulthood. However, the lifelong risk of the liver disease and its complications remains undisputable.

Diagnosis

Increased awareness of immunologists is a key to early detection of liver involvement. Routine biochemical blood tests and regular expert ultrasonography with stool screening for CS are recommended. Of note, standard stool microscopy could often miss protozoan infections, and therefore PCR-based techniques are preferable.

When CS is detected with whatever technique available, aggressive treatment is required. In immunodeficient patients, therapeutic options include nitazoxanide, paromomycin and azithromycin, often required in combination, in repeated courses or indefinitely [9, 14]. This clearly indicates that neither is very effective and that primary avoidance of this ubiquitous pathogen is recommended if possible. Thus, preventative measures such as drinking boiled water or azithromycin three times a week may be more appropriate in children diagnosed with conditions known to confer susceptibility to cholangiopathies such as hyper-IgM syndrome or DOCK-8 deficiency.

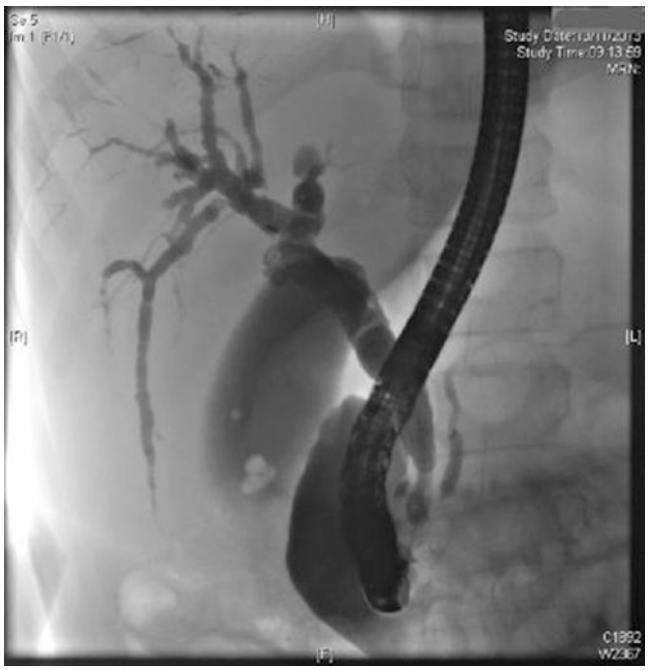

Fig. 65.1 Endoscopic retrograde cholangio-pancreatogram showing diffuse bilateral intra- and extrahepatic cholangiopathy in a child with a form of hyper-IgE syndrome (DOCK8 deficiency)

If prominence or dilatation of bile ducts is noted on ultrasound scan (USS), further imaging with magnetic resonance cholangiopancreatography (MRCP) is required. If MRCP is suggestive of dominant biliary stricture, endoscopic retrograde cholangiopancreatography (ERCP) could be indicated in order to attempt balloon dilatation, sample the bile for microbiology and biopsy the suspected lesion to rule out cholangiocarcinoma. Percutaneous liver biopsy under intravenous antibiotic cover is justified in the presence of consistent biochemical abnormality in patients with PIDs (see Fig. 65.1).

Management

Liver replacement will not work for the patients with PIDs until their immune defect is also rectified. There are many reports describing recurrence of cholangiopathy in the liver grafts within months after successful liver transplantation. Patients with less advanced liver disease can survive iso-

lated reduced-intensity conditioning (RIC) HSCT, but generally children with established cholangiopathies secondary to hyper-IgM syndrome, DOCK-8 deficiency or any other PIDs should be identified and screened early for a matched HSCT donor [15]. When the liver injury becomes clinically more severe, the children with PIDs are unlikely to tolerate conventional HSCT protocols, which often include potentially hepatotoxic medications. Thus, they should be transplanted while their liver involvement remains minimal, mild or absent if a good donor is available [16]. A sequential approach with combined liver and non-myeloablative RIC HSCT may be required for the ones with decompensated biliary cirrhosis and complications of advanced liver disease [17].

Effective HSCT not only prevents progression of established cholangiopathy but also could revert the histological abnormalities. We have observed four children with significant improvement of their histological features of cholangiopathy within couple of years after HSCT. However, there was no short-term change in the calibre of their bile ducts on ultrasonography [16].

Over the past decade, we have observed a reducing number of patients with PIDs and advanced chronic liver disease. There are two possible explanations for that: (1) these patients are referred earlier due to increased awareness of possible liver problems among immunologists, and (2) they receive their HSCT earlier, before serious liver disease develops. It is hoped that this strategy will continue to work and that the hepatologists would only exceptionally be required to see the patients with PIDs.

Haemophagocytic Lymphohistiocytosis

Haemophagocytic lymphohistiocytosis (HLH) is a condition which probably still remains underdiagnosed due to a relatively specialised diagnostic pathway and the clinical features mimicking acute septicaemia of early infancy. Majority of primary or familial HLH cases are a form of PID. Secondary or nonfamilial forms can be associated with any infectious trigger or presence of malignancy. In rheumatology, the term macrophage activation syndrome is used to highlight ubiquitous nature of this immune overreaction. A subgroup of patients with primary perforin deficiency presents acutely, often in infancy, with acute liver failure, but also in respiratory distress, fever, pancytopenia, rash and renal impairment, all indicative of systemic involvement. Perforin is a 60-kDa polypeptide, produced by cytoplasmic granules of activated natural killer (NK) cells and cytotoxic lymphocytes. Perforin physiologically assists in primary immune response by forming perforations/"pores" on membrane of the invading cells, facilitating entry of mediators such as granzyme, which lead to osmotic cell lysis and consequent cell death [18]. There are at least five different loci where mutations in perforin production pathway, leading to familial forms of HLH, can be detected [18].

The liver involvement at presentation is frequently so severe to warrant consideration for liver transplantation. In addition to classical signs of acute liver failure such as hyperbilirubinemia, coagulopathy and hypoalbuminaemia with ascites, those patients have hyperferritinaemia, hypertriglyceridaemia, hypofibrinogenaemia and signs of activated T lymphocytes, in particular interleukin (IL)-2R-positive cells in circulation. Patients often exhibit fever, splenomegaly and pancytopenia. Bone marrow aspirate reveals haemophygocytosis—a phenomenon defined by macrophages engulfing other cells and cellular debris. This urgent situation requires prompt identification of immune defect if it underlies the clinical situation, as liver transplantation would then only be a palliative measure; the underlying ongoing immune overactivity continues. Potential cure is control of the immune reaction by combination of steroids and chemotherapy (HLH protocol) [20]. One recently described novel option includes a selective blockage of proinflammatory cytokine pathway by using interferon-gamma blocking monoclonal antibody—emapalumab—for control of HLH-induced "cytokine storm" and its clinical consequences [21]. The overall mortality is high, and surviving patients should be worked up for HSCT as each new exposure to an infectious agent could trigger another life-threatening episode of haemophagocytosis [19].

Therefore, any young child who presents in indetermined acute liver failure with fever, splenomegaly and pancytopenia should, in addition to standard treatment of this condition, be urgently investigated for primary immunodeficiency by performing bone marrow aspirate and flow cytometry for expression of IL-2R and perforin on activated lymphocytes. Suggestion that the child could be immune deficient may indicate that consideration for emergency liver transplantation may not be justified as it could potentially result in a loss of precious liver graft.

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Autoimmune Liver Disease

66

Giorgina Mieli-Vergani and Diego Vergani

Autoimmune liver disorders are inflammatory diseases characterized histologically by a dense mononuclear cell infiltrate in the portal tract that invades the surrounding parenchyma (interface hepatitis, Fig. 66.1a), biochemically by increased levels of transaminases, and serologically by the presence of circulating autoantibodies and high levels of immunoglobulin G (IgG) [1]. These disorders usually respond to immunosuppressive treatment and can present insidiously or with a picture of acute hepatitis. Best results are obtained with early institution of immunosuppression [1–3].

There are three liver disorders in which liver damage is likely to arise from an autoimmune attack: autoimmune hepatitis (AIH), autoimmune sclerosing cholangitis (ASC), and de novo autoimmune hepatitis after liver transplant.

Hitherto considered rare, juvenile autoimmune liver diseases are reported more frequently than in the past, because of enhanced awareness, a real increase in their prevalence, and/or the decrease in viral hepatitis-related diseases.

Autoimmune Hepatitis

Clinical Features (Table 66.1)

Two forms of AIH are recognized according to the type of autoantibodies present in the serum. Type 1 AIH (AIH-1) is positive for smooth muscle antibody (SMA) and/or antinuclear antibody (ANA), while type 2 AIH (AIH-2) for anti-liver/kidney microsomal type 1 (anti LKM-1) and/or anti-liver cytosol type 1 (anti-LC-1) antibody (Fig. 66.2).

AIH-1 accounts for three-quarters of the cases and presents often around puberty, while AIH-2 tends to present at a younger age and also during infancy. IgG levels are usually raised at disease onset in both types, though 15% of children with AIH-1 and 25% of those with AIH-2 have normal values at presentation, particularly when the disease presents acutely [4, 5]. Partial IgA deficiency is common in AIH-2, affecting some 40% of patients [4, 6].

In children and adolescents, AIH has a more aggressive phenotype than in adult patients. For long, it was believed that children and adolescents of non-European origin were rarely affected by AIH and the clinical course of juvenile AIH has been mainly described in Caucasoid patients [3, 4, 7–13]. More recently, however, juvenile autoimmune liver diseases have been reported in several countries [14–24].

The mode of AIH presentation includes [3, 4, 7, 21, 25–27]:

- Acute presentation resembling that of viral hepatitis, with nonspecific symptoms of malaise, nausea/vomiting, anorexia, and joint and abdominal pain, accompanied by jaundice, dark urine, and pale stools (40–50% of patients with AIH-1 or AIH-2)
- Fulminant hepatic failure with grade II to IV hepatic encephalopathy developing 2 weeks to 2 months after the onset of symptoms (~3% of patients with AIH-1 and ~25% of patients with AIH-2)
- Insidious onset, characterized by nonspecific symptoms (progressive fatigue, amenorrhea, headache, anorexia, joint and abdominal pain, diarrhea, weight loss), lasting from 6 months to a few years before diagnosis (~40% of patients with AIH-1 and ~25% of patients with AIH-2)
- Late presentation with complications of cirrhosis and portal hypertension (hematemesis from esophageal/gastric varices, bleeding diathesis, splenomegaly), without previous history of jaundice or liver disease (~10 of both AIH types).
- Incidental finding of abnormal transaminase levels, without hepatic symptoms or signs (rare in older series but reported in some 30% of patients in a recent report [3]).

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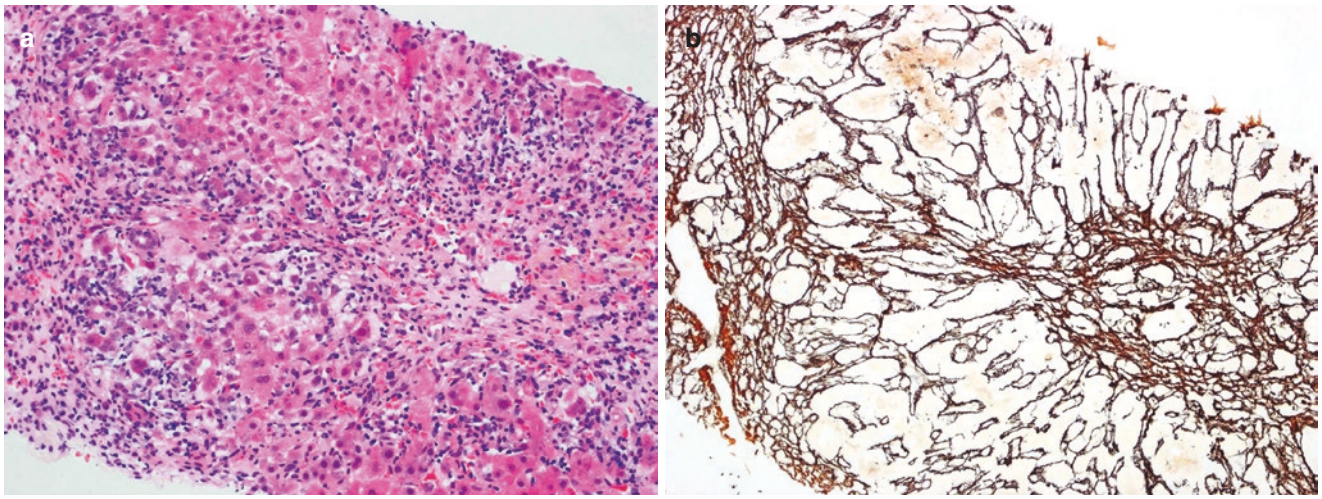

Fig. 66.1 Histological features of autoimmune hepatitis. (**a**) Portal and periportal lymphocyte and plasma cell infiltrate, extending to and disrupting the parenchymal limiting plate (interface hepatitis). Swollen hepatocytes, pyknotic necroses, and acinar inflammation are present. Hematoxylin and eosin staining. (**b**) Reticulin staining of the same biopsy showing connective tissue collapse resulting from hepatocyte death and expanding from the portal area into the lobule ("bridging collapse")

The mode of presentation of AIH in childhood is therefore variable, and the disease should be suspected and excluded in all children presenting with symptoms and signs of liver disease not ascribable to more common pathologies. The course of the disease can be fluctuating, with flares and spontaneous remissions, a pattern that may result in delayed referral and diagnosis. Often, however, on physical examination, the patients have clinical signs of an underlying chronic liver disease, including cutaneous stigmata (spider nevi, palmar erythema, leukonychia, striae), firm liver, and splenomegaly. At ultrasound, the liver parenchyma of these patients is often nodular and heterogeneous.

About one-third of patients with AIH have cirrhosis at the time of diagnosis, irrespective of the mode of presentation [3–5], indicating that the disease process is long-standing. AIH patients presenting acutely have often advanced fibrosis or cirrhosis on liver biopsy.

Severity of disease is similar in the two AIH types [3, 4, 28], but children with AIH-2 have higher levels of bilirubin and transaminases at onset than those who are ANA/SMA-positive, present more frequently with fulminant hepatic failure, and are more refractory to eventual treatment withdrawal [4, 20, 23]. Excluding children with the fulminant presentation, a severely impaired hepatic synthetic function, as indicated by the presence of prolonged prothrombin time and hypoalbuminemia, is more common in AIH-1 than in AIH-2. The severity of interface hepatitis at diagnosis is similar in both types, but cirrhosis on initial biopsy is more frequent in AIH-1 than in AIH-2, suggesting a more chronic course of disease in the former. Progression to cirrhosis during treatment is more frequent in AIH-1. In both types of AIH, a more severe disease course and a higher tendency to relapse are associated with the possession of antibodies to soluble liver antigen (SLA) [29, 30]. In both types, some 40% of patients have a family history of autoimmune disease [3, 4], and some 20–40% have themselves associated autoimmune disorders—including thyroiditis, type 1 diabetes, inflammatory bowel disease (IBD), hemolytic anemia, vitiligo, coeliac disease, Behçet disease, Sjögren syndrome, glomerulonephritis, idiopathic thrombocytopenia, urticaria pigmentosa, hypoparathyroidism, and Addison's disease (mainly in AIH-2) [3, 4, 31, 32] (Table 66.1). These associated conditions should be actively sought for prompt treatment [33]. In this context, diagnoses of particular importance are thyroiditis with hypothyroidism that affects 8–23% of patients [4, 31], coeliac disease that affects between 5% and 10% of patients [34–37], and IBD that is reported in 18% of patients [7]. Interestingly, patients with AIH and coeliac disease have been reported to achieve treatment-free sustained remission in a significantly higher proportion of cases than patients with AIH without coeliac disease, suggesting a possible long-term adjuvant effect of the gluten-free diet [38].

AIH-2 responsive to immunosuppressive treatment can be part of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome, an autosomal recessive genetic disorder characterized by the triad of chronic mucocutaneous candidiasis, hypoparathyroidism, and Addison's disease, in which AIH-2 is also present in some 20–30% of cases [39–41]. Autoimmune and immunodeficiency diseases reflect a dysfunctional immune system: multiple single-gene defects have been identified, resulting in rare diseases with features of both immunodeficiency and autoimmunity, including AIH [42–44].

As mentioned above, AIH is being increasingly reported in children and adolescents of non-Caucasoid descent, probably because the diagnosis of autoimmune liver disease was previously overlooked in view of the presence of epidemic

Table 66.1 Clinical presentation, histological features, and HLA association of childhood autoimmune liver disease: King's College Hospital experience [4, 7, 47]

Parameter	AIH-1	AIH-2	ASC
Median age in years	11	7	12
Mode of presentation (%)			
Acute hepatitis	47	40	37
Acute liver failure	3	25	0
Insidious onset	38	25	37
Complication of chronic liver disease	12	10	26
Associated autoimmune-disorders (%)	22	20	48
Inflammatory bowel disease (%)	20	12	44
Abnormal cholangiogram (%)	0	0	100
ANA/SMA (%)	100	25	96
Anti-LKM-1 (%)	0	100	4
pANNA (%)	45	11	74
Anti-SLA[a] (%)	58	58	41
Low C4 level (%)	89	83	70
Increased frequency of HLA DR*0301	Yes	No[b]	No
Increased frequency of HLA DR*0701	No	Yes	No
Increased frequency of HLA DR*1301	No	No	Yes
Interface hepatitis (%)			
any degree	92	94	60
moderate/severe	66	72	35
Histological biliary features (%)	28	6	35
Cirrhosis (%)	69	38	15

AIH autoimmune hepatitis, *ANA* antinuclear antibodies, *ASC* autoimmune sclerosing cholangitis, *SMA* anti-smooth muscle antibodies, *anti-LKM-1* anti-liver kidney microsomal type 1 antibody, *pANNA* peripheral antinuclear neutrophil antibodies, anti-*SLA* anti-soluble liver antigen, *IgG* immunoglobulin G, *C4* C4 component of complement, *HLA* human leukocyte antigen

[a]Measured by radioligand assay [29]

[b]But increased in HLA DR*0701-negative patients

viral hepatitis B and/or C. Reports from India [16, 21], Malaysia [17], Pakistan [15, 24], Bahrain [18], Iran [14], Egypt [23], Jamaica [22], and Mexico [20] indicate a clinical presentation and response to immunosuppressive treatment similar to those described in Caucasoid patients but an overall worse response to treatment and outcome, possibly related to delay in referral to specialized centers and diagnosis.

Epidemiology and Genetic Predisposition

The epidemiology of childhood autoimmune liver disease has not been studied in depth. Data collected at the King's College Hospital Pediatric Hepatology Tertiary Referral Center show a steady increase in the yearly incidence of juvenile autoimmune liver disease, only partially explained

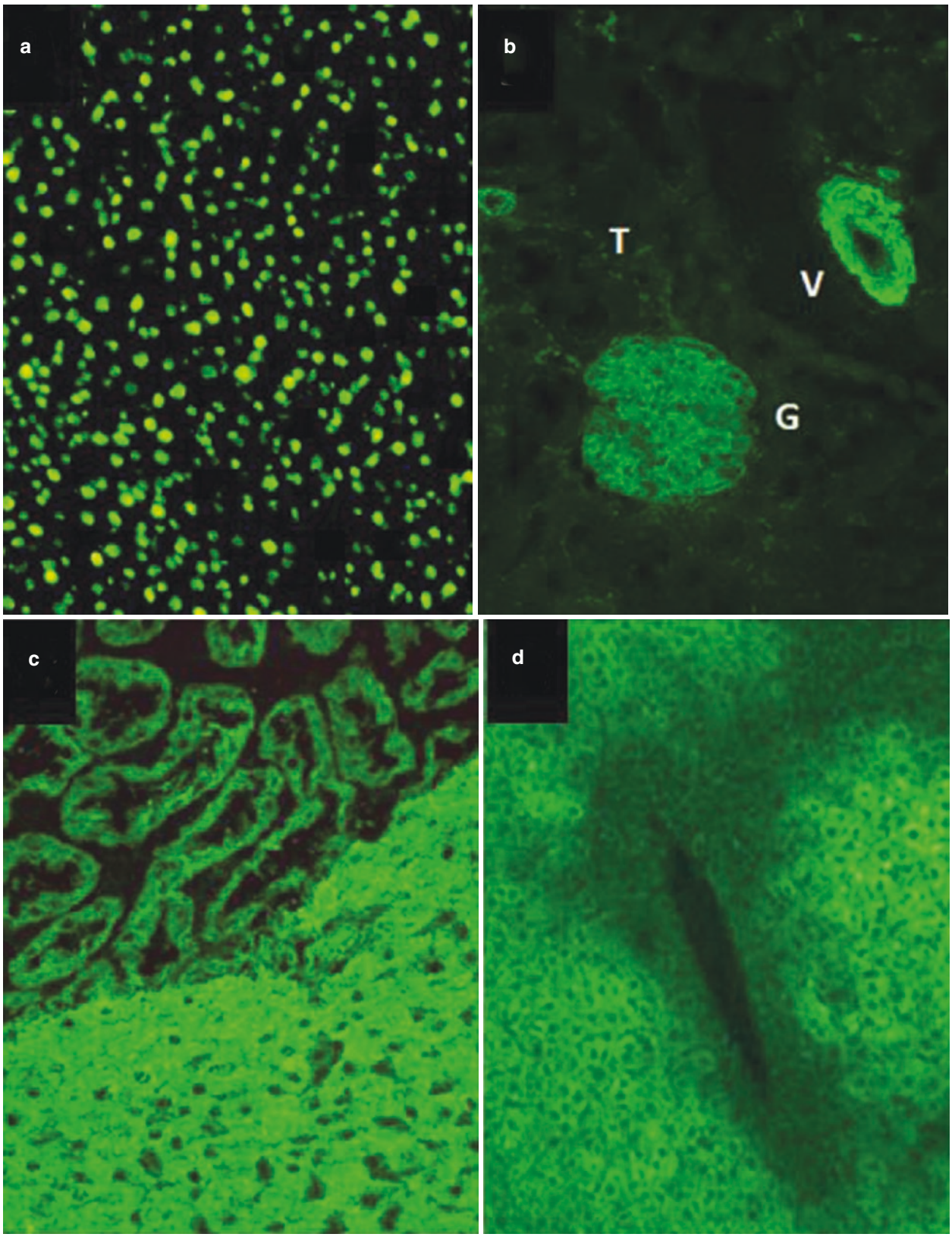

Fig. 66.2 Autoantibody immunofluorescence pattern on rodent tissue substrates. Panel (**a**) Antinuclear antibody (ANA) (liver): the homogeneous pattern is the most common in autoimmune hepatitis. Panel (**b**) smooth muscle antibody (SMA) (kidney): SMA stains the smooth muscle of arterial vessels (V), glomeruli (G), and tubules (T) (picture courtesy of Dr Luigi Muratori). Panel (**c**) anti-liver kidney microsomal type 1 antibody (LKM-1) (kidney and liver): anti-LKM-1 stains the cytoplasm of hepatocytes and proximal renal tubules. Panel (**d**) anti-liver cytosol type 1 (anti-LC-1) (liver): anti-LC-1 stains the cytoplasm of hepatocytes with a weakening of the stain around the central vein

by a referral bias: over the last 4 decades, the incidence has increased from 3.6 cases/year in the 1990s [4] to 5.4 cases/year in the 2000s [7] to 16.6 cases/year currently [3].

In Northern Europe, pediatric AIH-1, similar to adult AIH, is associated with the possession of the human leukocyte antigen (HLA) DRB1*03 (Table 66.1) [4, 45]. In contrast to adult patients, possession of DRB1*04 does not predispose to AIH in childhood and can even exert a protective role [4]. AIH-2 is associated with possession of DRB1*07 and, in DR7 negative patients, with possession of DRB1*03 (Table 66.1) [46, 47]. In Egypt, AIH-2 has also been associated with possession of HLA-DRB1*15 [48]. In Brazil and in Egypt, the primary susceptibility allele for AIH-1 is DRB1*1301, but a secondary association with DRB1*0301 has also been identified [48, 49]. Interestingly, in South America, possession of the HLA DRB1*1301 allele not only predisposes to pediatric AIH-1 but is also associated with persistent infection with the endemic hepatitis A virus [50, 51]. Pediatric patients with AIH, whether anti-LKM-1- or ANA/SMA-positive, have isolated partial deficiency of the HLA class III complement component C4, which is genetically determined [52].

As mentioned above, AIH-2 can be part of the APECED syndrome, in which the liver disease is reportedly present in some 20–30% of cases [39, 40].

Diagnosis

The diagnosis of AIH is based on a combination of clinical (see above), biochemical (raised serum transaminase levels), immunological (raised IgG levels, presence of autoantibodies), and histological indices and the exclusion of other known causes of liver disease that may share serological and histological features with AIH (e.g., hepatitis B, C, and E, Wilson's disease, nonalcoholic steatohepatitis, and drug-induced liver disease) [2, 53, 54].

Histology Liver biopsy is necessary to establish the diagnosis; the typical histological picture includes a dense mononuclear and plasma cell infiltration of the portal areas, which expands into the liver lobule leading to damage of the hepatocytes at the periphery of the lobule with erosion of the limiting plate ("interface hepatitis") (Fig. 66.1a). Hepatocytes surrounded by inflammatory cells become swollen and undergo pyknotic necrosis. Plasma cells are usually abundant at the interface and within the lobule, but their presence in low number does not exclude the diagnosis of AIH. When AIH presents acutely or at the time of relapse, panlobular hepatitis with connective tissue collapse resulting from hepatocyte death and expanding from the portal area into the lobule ("bridging collapse") is often observed (Fig. 66.1b). Nonspecific features that may point to the diagnosis of AIH are emperipolesis and hepatocyte rosetting [55]. The typical histological picture, however, is not always present at diagnosis as it varies according to disease stage or previous immunosuppressive treatment for associated conditions [56]. It has been suggested that in pediatric AIH hyaline droplets in Kupffer cells might be a useful diagnostic marker to distinguish AIH from other forms of chronic hepatitis, the hyaline droplets being positive for IgG by immunohistochemistry and correlating with a >twofold increase in serum level of IgG [57].

Histology also allows evaluating the extent of fibrosis and helps in identifying overlap syndromes or possible presence of concomitant diseases, such as nonalcoholic fatty liver disease [58]. Though inflammatory changes surrounding the bile ducts have been reported also in a proportion of patients with classical AIH [59], when conspicuous they suggest an overlap with sclerosing cholangitis.

Autoantibodies A key diagnostic criterion for all AIH scoring systems is the detection of autoantibodies (ANA, SMA, anti-LKM-1 and anti-LC-1) [60, 61], which not only assists in the diagnosis but also allows differentiation of AIH types. ANA and SMA characterize AIH-1, while anti-LKM-1 and anti-LC-1define AIH-2, though occasionally ANA or SMA can coexist with anti-LKM-1 or anti-LC-1, the clinical course in these cases being similar to that of AIH-2. A major target of SMA is the actin of smooth muscle, whereas the molecular target of LKM-1 is cytochrome P-4502D6 (CYP2D6) [62] and of anti-LC-1 is formiminotransferase cyclodeaminase [63]. ANA, SMA, anti-LKM-1, and anti-LC-1 should be sought by indirect immunofluorescence using rodent stomach, kidney, and liver as substrate, as other techniques, e.g., commercially available enzyme linked immunosorbent assays (ELISAs), remain to be fully validated (Fig. 66.2) [60]. In contrast to adults, in healthy children, autoantibody reactivity is infrequent, so that titers of 1:20 for ANA and SMA and 1:10 for anti-LKM-1 are clinically relevant. Anti-LC-1, also detectable by indirect immunofluorescence, can be present on its own but frequently occurs in association with anti-LKM-1. This co-occurrence can go unnoticed because anti-LKM-1 obscures the anti-LC-1 pattern. Anti-LC-1 can also be detected by commercial available tests (ELISAs, line blots, and immunoblots). Positivity for autoantibodies is not sufficient for the diagnosis of AIH since they can be present, usually at low titer, in other liver disorders such as viral hepatitides [64, 65], Wilson's disease [66], and nonalcoholic steatohepatitis [67].

Other autoantibodies less commonly tested but of diagnostic importance include peripheral antinuclear neutrophil antibody (atypical pANCA or pANNA) and anti-SLA. pANNA is frequently found in AIH-1 and in ASC and is also common in IBD, while it is virtually absent in AIH-2. Anti-SLA, originally described as the hallmark of a third type of AIH [68], is also found in up to 50% of patients with AIH-1,

AIH-2, or ASC, where it defines a more severe course [29, 30]. Anti-SLA is not detectable by conventional immunofluorescence, but the definition of its molecular target as UGA transfer RNA (tRNA) suppressor-associated antigenic protein (SepSecS) [69, 70] has enabled the establishment of molecularly based diagnostic assays. However, it should be noted that ELISAs are less sensitive than radioligand assays available in research laboratories.

There are a small proportion of patients with AIH without detectable autoantibodies. This condition, which responds to immunosuppression like the seropositive form, represents seronegative AIH [71], a rare type of AIH in adults, whose prevalence and clinical characteristics remain to be defined in children.

Diagnostic Criteria The diagnosis of AIH has been advanced by the scoring systems developed by the International Autoimmune Hepatitis Group (IAIHG) for adult patients [53, 54] where negative criteria such as evidence of infection with hepatitis B or C virus, Wilson's disease, or alcoholic liver disease, among others, are taken into account in addition to the positive criteria mentioned above. The IAIHG scoring system was devised mainly for research purposes to allow ready comparison between series from different centers but has also been used clinically, including in pediatric series. More recently, the IAIHG have published a simplified scoring system based on autoantibodies, IgG, histology, and exclusion of viral hepatitis that is better suited to clinical application [72]. However, neither scoring system is suitable to the juvenile form of the disease, where diagnostically relevant autoantibodies often have titers lower than the cutoff value considered positive in adults [73–75]. In addition, neither system can distinguish between AIH and ASC (see below) [7, 76], which can only be differentiated if a cholangiogram is performed at presentation. For this reason, the European Society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) has published in 2018 a position paper for the diagnosis and management of pediatric autoimmune liver disease, which includes a diagnostic scoring system that should be evaluated in a large number of patients with juvenile AIH or ASC for validation [2]. Not included in the ESPGHAN criteria, but useful in the differentiation between AIH and ASC, is the alkaline phosphatase/aspartate aminotransferase ratio, which is significantly higher in ASC than in AIH, a ratio ≥ 3 being strongly suggestive of ASC [3, 7].

Pathophysiology

The typical histological picture of AIH, which is characterized by a dense mononuclear cell infiltrate eroding the limiting plate and invading the parenchyma (interface hepatitis, Fig. 66.1a), first suggested that autoaggressive cellular immunity might be involved in its causation [77]. Immunocytochemical studies have identified the phenotype of the infiltrating cells. T lymphocytes mounting the alpha/beta T-cell receptor predominate. Among the T-cells, a majority is positive for the CD4 helper/inducer phenotype, and a sizable minority is positive for the CD8 cytotoxic phenotype. Lymphocytes of non-T-cell lineage are fewer and include (in decreasing order of frequency) natural killer cells (CD16/CD56-positive), macrophages, and B lymphocytes [78, 79]. Natural killer T-cells, which express simultaneously markers of both natural killer (CD56) and T-cells (CD3), are involved in liver damage in an animal model of autoimmune hepatitis [79].

A powerful stimulus must be promoting the formation of the massive inflammatory cell infiltrate present at diagnosis. Whatever the initial trigger, it is most probable that such a high number of activated inflammatory cells cause liver damage. There are different possible pathways that an immune attack can follow to inflict damage on the hepatocyte (Fig. 66.3). Liver damage is believed to be orchestrated by CD4-positive T lymphocytes recognizing a self-antigenic peptide. To trigger an autoimmune response, the peptide must be embraced by an HLA class II molecule and presented to uncommitted T helper (Th0) cells by professional antigen-presenting cells (APCs), with the co-stimulation of ligand–ligand (CD28 on Th0, CD80 on APC) interaction between the two cells. The Th0 cells become activated, differentiate into functional phenotypes according to the cytokines prevailing in the microenvironment and the nature of the antigen, and initiate a cascade of immune reactions determined by the cytokines they produce. Arising in the presence of the macrophage-produced interleukin-12, Th1 cells secrete mainly interleukin-2 and interferon-gamma, which activate macrophages, enhance expression of HLA class I (increasing the vulnerability of liver cells to cytotoxic attack), and induce expression of HLA class II molecules on hepatocytes, which then become able to present the autoantigenic peptide to Th cells, thus perpetuating the immune recognition cycle. Th2 cells, which differentiate from Th0 if the microenvironment is rich in interleukin-4, produce mainly interleukin-4, interleukin-5, and interleukin-10, which induce autoantibody production by B lymphocytes. Physiologically, Th1 and Th2 cells antagonize each other. The process of autoantigen recognition is strictly controlled by regulatory mechanisms. If these regulatory mechanisms fail, the autoimmune attack is perpetuated. Over the past four decades, different aspects of the above pathogenic scenario have been investigated.

An impairment of immunoregulatory mechanisms has been described in AIH. Both children and young adults with this condition have low levels of T-cells expressing the CD8 marker and impaired suppressor cell function [80, 81] which

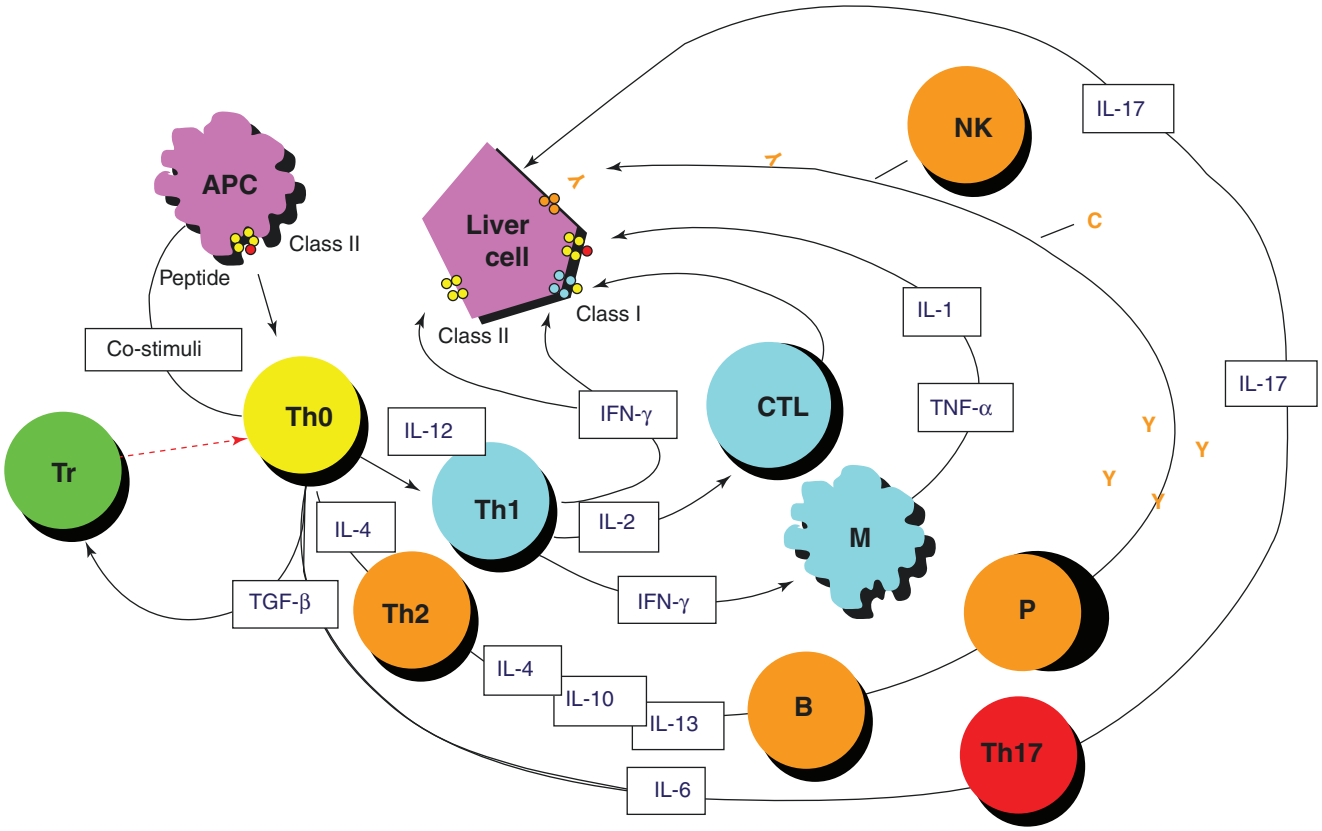

Fig. 66.3 An autoantigen is presented to uncommitted T helper (TH0) lymphocytes within the HLA class II molecule of an antigen-presenting cell (APC) either in the regional lymph nodes or within the liver itself. Activated TH0 cells differentiate into TH1 or TH2 cells in the presence of interleukin (IL)-12 or IL-4 and according to the nature of the antigen and trigger a series of immune reactions determined by the cytokines they produce: TH1 cells secrete IL-2 and interferon-gamma (IFN-γ), cytokines that stimulate cytotoxic T lymphocytes (CTL), enhance expression of class I and induce expression of class II HLA molecules on the liver cells, and activate macrophages. The latter release IL-1 and tumor necrosis factor alpha (TNF-α). TH2 cells secrete mainly IL-4, IL-10, and IL-13 and stimulate autoantibody production by B lymphocytes. In the presence of defective regulatory T-cells (Treg), hepatocyte destruction ensues from the engagement of damaging effector mechanisms, including CTL, cytokines released by TH1 and activated macrophages, complement activation, as well as adhesion of natural killer (NK) cells to autoantibodycoated hepatocytes through their Fc receptors. TH17 cells, a recently described proinflammatory population that derives from TH0 cells in the presence of transforming growth factor beta (TGF-β) and IL-6, are focus of current investigation

segregates with the possession of the HLA haplotype B*08/DRB1*03 (formerly B8/DR3) and can be corrected by therapeutic doses of corticosteroids [82]. Moreover, patients with AIH have been reported to have a specific defect in a subpopulation of T-cells controlling the immune response to liver-specific membrane antigens [83]. Further evidence for an impairment of immunoregulatory function in AIH has been obtained in the last two decades [84–86]. Among T-cell subsets with potential immunosuppressive function, CD4+ T-cells constitutively expressing the interleukin 2 receptor (IL-2R) alpha chain (CD25) (regulatory T-cells, Tregs) have emerged as the dominant immunoregulatory population [87]. These cells, which represent 5–10% of the total population of peripheral CD4+ T lymphocytes, control the innate and the adaptive immune responses by preventing the proliferation and effector function of autoreactive T-cells. Their mechanism of action involves mainly a direct contact with the target cells and to a lesser extent the release of immunoregulatory cytokines, such as interleukin 10 and tissue growth factor beta 1.

A numerical Treg impairment affects both children and adults with AIH. This defect is more evident at diagnosis than during drug induced remission, although even then circulating Treg frequencies fail to reach the levels seen in health [84, 88, 89]. The percentage of Tregs inversely correlates with biomarkers of disease severity, suggesting that a reduction in regulatory T-cells favors autoimmune liver disease and its severity. Importantly, Tregs from AIH patients at diagnosis are impaired in their ability to control the proliferation of CD4 and CD8 effector cells compared to Tregs isolated from AIH patients at remission or from healthy subjects [88].

Effector CD4 T-cells isolated from patients with AIH are less susceptible to the regulatory control exerted by Tregs. This defect is linked to reduced expression of the inhibitory receptor T-cell-immunoglobulin- and-mucin-

domain-containing molecule-3 (Tim-3), which upon ligation of galectin-9 expressed by Tregs induces effector cell death [90].

Hepatocytes from patients with AIH, in contrast to normal hepatocytes, express HLA class II molecules [91], and, although lacking the antigen-processing machinery typical of APCs, they may present peptides through a bystander mechanism. Given the impaired regulatory function and the inappropriate expression of HLA class II antigens on the hepatocytes, it is conceivable that an autoantigenic peptide is presented to the helper/inducer cells, leading to their activation. Although there is no direct evidence as yet that an autoantigenic peptide is presented and recognized, activation of helper cells has been documented in AIH [78, 92]. These activated cells possess the CD4 phenotype, and their numbers are highest when the disease is most active.

Most advances in the study of T-cells have occurred in AIH-2, since the knowledge that CYP2D6 is the main autoantigen has enabled the characterization of both CD4 and CD8 T-cells targeting this cytochrome. One study has shown that CD4 T-cells from patients with AIH-2 positive for the predisposing HLA allele DRB1*0701 recognize seven regions of CYP2D6 [46], five of which have later been shown to be also recognized by CD8 T-cells [93]. High numbers of interferon-gamma-producing CD4 T-cells and CD8 T-cells are associated with biochemical evidence of liver damage, suggesting a combined cellular immune attack.

What triggers the immune system to react to an autoantigen is unknown. A lesson may be learned by the study of humoral autoimmune responses during viral infections. Thus, studies aimed at determining the specificity of the LKM-1 antibody, present in both the juvenile form of AIH and in some patients with chronic HCV infection, have shown a high amino acid sequence homology between the HCV polyprotein and CYP2D6, the molecular target of anti-LKM-1, thus implicating a mechanism of molecular mimicry as a trigger for the production of anti LKM-1 in HCV infection [62, 94, 95]. It is therefore conceivable that an as yet unknown virus infection may be at the origin of the autoimmune attack in AIH.

Titers of antibodies to liver-specific lipoprotein, a macromolecular complex present on the hepatocyte membrane, and to its well-characterized component asialoglycoprotein receptor correlate with the biochemical and histologic severity of AIH [96, 97]. Antibodies to alcohol dehydrogenase, a second well-defined component of liver-specific lipoprotein, have been described in patients with AIH [98]. Immunofluorescence studies on monodispersed suspensions of liver cells obtained from patients with AIH showed that these cells are coated with antibodies in vivo [99]. A pathogenic role for these autoantibodies has been indicated by cytotoxicity assays showing that autoantibody-coated hepatocytes from patients with AIH are killed when incubated with autologous lymphocytes. The effector cell was identified as an Fc receptor-positive mononuclear cell [100]. T-cell clones obtained from liver biopsies of children with AIH and expressing the gamma/delta T-cell receptor have been shown to be cytotoxic to a variety of targets but to preferentially kill liver-derived cells as opposed to cell lines derived from other organs [101].

The establishment of cell lines and clones has shown that the majority of T-cell clones obtained from the peripheral blood and a proportion of those from the liver of patients with AIH are CD4-positive and use the conventional alpha/beta T-cell receptor [101–104]. Some of these CD4-positive clones were further characterized and were found to react with partially purified antigens, such as crude preparations of liver cell membrane or liver-specific lipoprotein [102], and with purified asialoglycoprotein receptor [102, 104], or recombinant CYP2D6 [103] and to be restricted by HLA class II molecules in their response. Because CD4 is the phenotype of Th cells, T-cell clones were investigated for their ability to help autologous B lymphocytes in the production of immunoglobulin in vitro [102, 104]. Indeed, their coculture with B lymphocytes resulted in a dramatic increase in autoantibody production.

The possible role of Th17 cells in the pathogenesis of AIH is under investigation. Th17 cells contribute to autoimmunity by producing the proinflammatory cytokines IL-17, IL-22, and TNF-α and inducing hepatocytes to secrete IL-6 [105], which further enhances Th17 activation. Th17 cells have been shown to be elevated in the circulation and liver of patients with AIH [105].

Treatment

The aim of treatment is to obtain a complete biochemical, immunological, and clinical remission in order to halt the progression of the disease.

Definition of Remission/Relapse For juvenile autoimmune liver disease, remission is defined as complete clinical recovery with transaminase levels within the normal range. It is obtained in 60–90% of patients [4, 14, 16, 23, 26], the rapidity and degree of treatment response depending on disease severity. More recently, three more remission criteria have been added: normalization of IgG levels, negative or very low titer autoantibodies, and histological resolution of inflammation [2, 5]. Histological improvement, however, lags behind clinical, biochemical, and immunological response [106–108], though after a mean duration of 4 years of effective treatment, some 95% of patients show marked histological amelioration [107]. As liver biopsy cannot be repeated frequently, for clinical pur-

poses, remission is considered complete when transaminase and IgG levels are normal, ANA and SMA are negative or low titer (<1:20), and anti-LKM1 and anti-LC1 are <1:10 or negative [2].

Relapse is characterized by increase of serum transaminase levels after remission has been achieved. Relapse during treatment occurs in some 40% of patients and requires a temporary increase of the steroid dose. An important cause of relapse is nonadherence, which is common, particularly in adolescents [3, 17, 109]. In more aggressive cases, the risk of relapse is higher if steroids are administered on an alternate-day schedule, which is often instituted in the assumption that may have a less negative effect on the child's growth. Small daily doses, however, are more effective in maintaining disease control, avoiding adverse effects, and minimizing the need for high-dose steroid pulses during relapses (with attendant more severe side effects).

Standard Treatment When AIH presents with fulminant hepatic failure (i.e., acute liver failure and encephalopathy), it usually requires urgent transplantation, though patients may respond to a trial of steroids, avoiding transplantation [25]. With all other modes of presentation, AIH responds satisfactorily to immunosuppression, even in the presence of poor synthetic function and/or established cirrhosis [3, 4, 7]. Treatment should be started with prednisolone 2 mg/g/d (maximum 60 mg/d), which is gradually decreased over a period of 4–8 weeks if there is progressive normalization of the transaminases, and then the patient is maintained on the minimal dose able to sustain normal transaminase levels, usually 5 mg/d. During the first 6–8 weeks of treatment, liver function tests should be checked weekly to allow a frequent fine-tuning of the treatment, avoiding severe steroid side effects. The initial goal is to obtain at least 80% reduction of the transaminase levels by 8 weeks of treatment. During this period of time, if progressive normalization of the liver function is not observed at weekly blood tests, azathioprine is added at a starting dose of 0.5 mg/g/d, which, in the absence of signs of toxicity, is gradually increased up to a maximum of 2–2.5 mg/g/d until remission (i.e., normal transaminase levels) is achieved [2]. Azathioprine is not recommended as first-line treatment because of its potential hepatotoxicity, particularly in severely jaundiced patients. Interestingly, it has been shown that neither thiopurine methyltransferase genotype nor activity predicts azathioprine hepatotoxicity in AIH, which appears instead to be related to the degree of liver fibrosis [110]. Measurement of the azathioprine metabolites 6-thioguanine (6-TGN) and 6-methylmercaptopurine has been reported to help in identifying drug toxicity and nonadherence and in achieving a level of 6-thioguanine considered therapeutic for inflammatory bowel disease [111], though an ideal therapeutic level for AIH has not been determined. In a retrospective review, 87% of 66 children with AIH were reported to maintain sustained biochemical remission (normal transaminase levels) in association with low 6-TGN levels ranging from 50 to 250 pmol on an azathioprine dose of 1.2–1.6 mg/kg/day [112]. Although an 80% decrease of initial transaminase levels is usually obtained within 6–8 weeks from starting treatment in most patients, complete normalization of liver function may take several months [3, 4]. Using the above schedule, steroid side effects are usually mild, the only serious complication being psychosis during induction of remission in 4%, which resolves after prednisolone withdrawal [4]. All patients develop a transient increase in appetite and mild cushingoid features during the first few weeks of treatment. After 5 years of treatment, 56% of our patients maintained their baseline centile for height or went up across a centile line, 38% dropped across one centile line, and only 6% dropped across two centile lines [113]. Moreover, all patients achieved their expected final height after a median steroid treatment of 8.9 years [113].

Treatment should be continued for at least 2–3 years before considering its cessation, after which period stopping treatment can be attempted but only if liver function tests and IgG levels have been persistently normal, and autoantibodies are either undetectable or detectable at very low titer (ANA/SMA <1:10; anti-LKM-1 should be negative) over at least 12 months, and a liver biopsy shows no inflammatory changes [2]. However, it is advisable not to attempt treatment withdrawal during or immediately before puberty, when relapses are more common. Treatment withdrawal is reportedly successful in 20% of children with AIH-1 but is rarely achieved in AIH-2 [3, 4, 9]. An important role in monitoring the response to treatment is the measurement of IgG levels and autoantibody titers, the fluctuation of which is correlated with disease activity [114].

A few studies using quality of life questionnaires report a decreased health-related score in young patients with AIH compared to healthy controls, advanced disease, active symptoms, extrahepatic autoimmune diseases, and a dislike of taking medications being associated with the worse scores [115–117]. However, a recent series of 83 patients with AIH or ASC followed up long term shows an excellent real-life clinical, educational, and social outcome in those who respond to immunosuppressive treatment [3]. Development of end-stage liver disease requiring liver transplantation 3–14 years after diagnosis, despite treatment, has been reported in 8–16% of children with AIH, the need for transplant being correlated to poor response to treatment, episodes of relapse, length of follow-up, and a diagnosis of ASC [3, 4, 7].

Maintenance with azathioprine monotherapy has been advocated once remission is achieved [112, 118], but whether this is effective long term and whether it offers any benefit on possible side effects compared to low-dose prednisolone/azathioprine maintenance is unclear.

Alternative Treatments

Induction of Remission Budesonide would appear to be an attractive drug for the induction and maintenance of remission in AIH, as its hepatic first-pass clearance is >90% of the oral dose and it has fewer side effects than predniso(lo)ne [119]. Budesonide, however, cannot be used in the presence of cirrhosis, which affects at least a third of AIH patients. In a large European study including adults and children with AIH, a combination of budesonide and azathioprine was reported to have fewer adverse effects compared to medium-dose standard prednisone and azathioprine [120]. In that study, budesonide at a dose of 3 mg three times daily, decreased upon response, was compared with prednisone 40 mg once daily reduced per protocol irrespective of response. After 6 months of treatment, remission was achieved in 60% of the budesonide group but in only 39% of the prednisone group, both percentages being worse than those achieved with standard treatment [3, 4, 7]. The results among the children recruited into that study were particularly disappointing, with a similarly low remission rate of 16% for budesonide and 15% for prednisone after 6 months of treatment and of 50% and 42%, respectively, after 12 months of treatment, with similar steroid side effects in both groups, apart from higher frequency of weight gain in children on prednisone [121]. Large controlled studies are needed to establish the appropriate dose for children and to evaluate whether budesonide has any advantage over predniso(lo)ne [122].

Induction of remission has been obtained in 72% treatment-naïve children with AIH-1 using cyclosporine A alone for six months, followed by maintenance with low-dose prednisone and azathioprine [123]. A 5-year follow-up of this study shows that 94% of the patients eventually achieved remission, with minor side effects [124]. Whether this mode of induction has any advantage over the standard treatment remains to be evaluated in controlled studies in specialized centers.

Tacrolimus is a more potent immunosuppressive agent than cyclosporine, but it also has significant toxicity. There is limited evidence supporting its role as treatment of AIH. Its use in the juvenile form of the disease is limited to one report, where tacrolimus was administered to 17 children with newly diagnosed AIH with or without the addition of prednisolone and/or azathioprine and to 3 children who had failed conventional therapy. Target tacrolimus trough levels were relatively low (2.5–5 ng/ml) and similar to those used in the maintenance of successful liver transplant. The study shows that monotherapy with tacrolimus was not sufficient to achieve complete remission in most cases, but the calcineurin inhibitor allowed reduction of the dose of prednisolone and azathioprine. On the other hand, tacrolimus-related side effects were noted in half of the patients [125].

Difficult-to-Treat Patients In those patients (up to 10%) in whom standard immunosuppression is unable to induce stable remission or who are intolerant to azathioprine, mycophenolate mofetil at a dose of 20 mg/kg twice daily together with prednisolone has been successfully used [126]. A meta-analysis, including data from several small studies of second-line treatments in children with AIH refractory to standard therapy, suggests that calcineurin inhibitors might have the highest response rate at 6 months but also the highest rate of adverse events; MMF was the second most effective drug with a low side effect profile, indicating that MMF is currently the best choice for second-line therapy [127]. If the absence of response persists or if there is intolerance to MMF (headache, diarrhea, nausea, dizziness, hair loss, and neutropenia), calcineurin inhibitors should be considered.

There is anecdotal experience with the successful use of the anti-B lymphocyte monoclonal antibody rituximab in two children with refractory AIH [128]. However, despite the relatively low adverse event profile of the drug, its use has been associated with a 2.4% rate of sepsis in children with autoimmune diseases [129].

Infliximab has been reported to be effective in the treatment of refractory AIH, including in a pediatric case [130–132], though it has potential serious side effects, including infections and hepatotoxicity [132]. In addition, anti-TNF-α-induced AIH has been described both in adults and children treated for IBD or other autoimmune conditions [133–135]. A better understanding of the role of TNF-α in the pathogenesis of AIH is needed before recommending its use in AIH.

As patients with AIH have a defect in immunoregulation affecting regulatory T-cells, sirolimus, a drug that expands selectively Tregs in vivo and in vitro [136], was used in four patients with refractory AIH, with short-term beneficial effect in two [137].

Of note, a survey on the management of juvenile AIH commissioned by the IAIHG [138] has shown that within the pediatric IAIHG members, there is considerable more experience with second-line therapeutic agents than among the IAIHG adult hepatologist members [139].

As mentioned above, children who present with fulminant hepatic failure, i.e., with grade 3–4 encephalopathy and INR ≥2, pose a particularly difficult therapeutic problem.

Although it has been reported that they may benefit from conventional immunosuppressive therapy [25, 140, 141], most require liver transplantation [4]. Encouraging results have been reported using cyclosporine A in anti-LKM-1-positive patients presenting with fulminant hepatitis [140]. These treatments should be evaluated on a larger number of patients because our own experience has not confirmed the value of this therapeutic approach.

Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED)

APECED is a monogenic disorder due to mutations of the *AIRE* gene [142, 143]. Its phenotype is variable and includes AIH in about 20–30% of the cases [39, 40]. This resembles AIH-2 and responds to standard immunosuppressive treatment [39], though the anti-LKM-1-like antibody detected by immunofluorescence targets cytochromes P450-2A1 and P450-2A6 [40], besides P450-2D6 [144]. APECED, also known as autoimmune polyendocrine syndrome 1, is usually an autosomal recessive disorder caused by homozygous mutations in the *AIRE* gene and characterized by a variety of organ-specific autoimmune diseases, the most common of which are hypoparathyroidism and primary adrenocortical failure, accompanied by chronic mucocutaneous candidiasis. The presence of two of these conditions is deemed to be diagnostic, though more recently diagnosis has been based on a combination of genetic and immunological tests [145]. Occasionally, depending on the genetic abnormality, the condition has been reported to be inherited in a dominant fashion [146].

To date, over 115 *AIRE* mutations have been identified in the APECED syndrome [147], present both in coding and noncoding regions and generating truncated protein sequences or single amino acid missense variants. These variants include single-nucleotide substitutions, insertions, and deletions with protein splicing effects [148, 149].

The protein encoded by *AIRE* is a transcription factor. *AIRE* is highly expressed in medullary epithelial cells and other stromal cells in the thymus involved in clonal deletion of self-reactive T-cells. Studies in a murine model indicate that the gene inhibits organ-specific autoimmunity by inducing thymic expression of peripheral antigens in the medulla leading to central deletion of autoreactive T-cells. Though the inheritance pattern of APECED indicates a recessive disorder, there are anecdotal data of mutations in a single copy of *AIRE* being associated with autoimmune disease of a less severe form than classically defined APECED [142, 143]. The role of *AIRE* heterozygote state in the development of AIH remains to be established. *AIRE* mutations have been reported in three children with severe AIH type 2 and extrahepatic autoimmune manifestations [150].

Autoimmune Sclerosing Cholangitis

Clinical Features

Sclerosing cholangitis is an uncommon disorder, characterized by chronic inflammation and fibrosis of the intrahepatic and/or extrahepatic bile ducts. When bile duct damage is detectable histologically, but cholangiography is normal, a diagnosis of "small duct disease" is made. In childhood, sclerosing cholangitis may occur as an individual disease or may develop in association with a wide variety of disorders. The term primary sclerosing cholangitis (PSC), used in adult patients, is not accurate to describe pediatric sclerosing cholangitis: "primary" denotes ignorance about etiology and pathogenesis, while in pediatrics there are etiologically well-defined forms of sclerosing cholangitis [7, 10, 151–154]. In the neonatal period, pathological features of severe sclerosing cholangitis characterize biliary atresia as well as neonatal sclerosing cholangitis, a condition inherited in an autosomal recessive manner [155]. Some other inherited diseases and immunological defects may produce a clinical picture similar to adult PSC. For example, mild to moderate defects in the *ABCB4* (MDR3) gene are a likely cause of a number of cases of small duct PSC in children and adults [156, 157], and sclerosing cholangitis may complicate a wide variety of disorders, including primary and secondary immunodeficiencies, Langerhans cell histiocytosis, psoriasis, cystic fibrosis, reticulum cell sarcoma, and sickle cell anemia. Moreover, an overlap syndrome between AIH and sclerosing cholangitis (autoimmune sclerosing cholangitis, ASC) is significantly more common in children than in adults [158]. In only a relatively small number of pediatric patients, sclerosing cholangitis occurs without any of the above defining features. The term of PSC would be better confined to the latter, as different types of sclerosing cholangitis respond to different therapeutic managements.

ASC is characterized by florid autoimmune features, positive autoantibodies, especially ANA and SMA, hypergammaglobulinemia, and interface hepatitis on liver biopsy [7, 158, 159].

Since these features are shared with AIH, and alkaline phosphatase (AP) or gamma-glutamyl transpeptidase (GGT) levels are often not elevated at disease onset, the diagnosis of ASC relies on the findings of bile duct damage on cholangiographic studies. In a 16-year prospective study, during which all children with serological and histological features of AILD underwent liver biopsy, sigmoidoscopy, and cholangiography at presentation [7], approximately half were found to have bile duct changes characteristic of sclerosing cholangitis (Fig. 66.4) and were therefore diagnosed with ASC. Importantly, a quarter of children with ASC had no histological features pointing to bile duct involvement, despite

Fig. 66.4 Magnetic resonance cholangiography of a child with autoimmune sclerosing cholangitis showing a diffuse cholangiopathy with strictures and dilatations affecting the intrahepatic bile ducts

abnormal cholangiograms. Virtually all ASC patients were seropositive for ANA and/or SMA. In contrast to AIH, which is a predominantly a disease of females, ASC affects similarly boys and girls [3, 7].

The mode of presentation of ASC is similar to that of AIH-1, although an association with IBD is more common in ASC (45–73%) than AIH-1 (11–20%) [3, 7]. Of note, in the King's prospective study [7], only one-third of the children with autoimmune liver disease and IBD had bowel symptoms, while the others were diagnosed because of surveillance sigmoidoscopy. At presentation, liver function tests—including GGT levels, which are a more reliable indicator of cholestasis than AP in growing children/adolescents, in whom AP often reflects bone growth—do not discriminate between AIH and ASC, though the AP/AST ratio is significantly higher in ASC (Table 66.2). Notably, pANNA positivity is present in 74% of children with ASC but only 45% of those with AIH-1 and 11% of those with AIH-2 [7]. Anti-SLA by a sensitive radioligand assay was found in some 50% of patients with ASC, and also in this condition it defines a more severe disease course [29]. HLA studies have shown that in the UK susceptibility to ASC is conferred by the possession of HLA DRB1*1301 and DRB1*0301 [47].

Evolution from AIH to ASC was documented in one patient during the published prospective series [7] and has been observed in two further patients during follow-up [160]. This was also documented in a recent retrospective review of the long-term outcome of juvenile AIH and ASC [3], suggesting that AIH and ASC may be part of the same pathologic process. Clinical, laboratory, and histological features of type 1 and 2 AIH and ASC are compared in Tables 66.1 and 66.2.

Table 66.2 Biochemical findings at presentation in childhood autoimmune liver disease: King's College Hospital experience [4, 7]. Results expressed as median (range)

	AIH	ASC
Bilirubin (nv <20 μmol/L)	35 (4–306)	20 (4–179)
Albumin (nv >35 g/L)	35 (25–47)	39 (27–54)
AST (nv <50 IU/L)	333 (24–4830)	102 (18–1215)
INR (nv <1.2)	1.2 (0.96–2.5)	1.1 (0.9–1.6)
GGT (nv <50 IU/L)	76 (29–383)	129 (13–948)
AP (nv <350 IU/L)	356 (131–878)	303 (104–1710)
AP/AST ratio	1.14 (0.05–14.75)	3.96 (0.20–14.20)

AIH autoimmune hepatitis, *ASC* autoimmune sclerosing cholangitis, *AST* aspartate aminotransferase, *INR* international normalized ratio, *GGT* gamma-glutamyl transpeptidase, *AP* alkaline phosphatase, *nv* normal values

Though "small duct disease" is reported rarely in pediatric series, in a study [153], where cholangiography was mainly performed by magnetic resonance (MRCP), no radiological biliary involvement was detected despite histological evidence of sclerosing cholangitis in a high proportion (36%) of patients, some of whom had autoimmune features. Whether this finding is due to a lower sensitivity of the MRCP compared to retrograde cholangiopancreatography in detecting biliary changes remains to be verified.

Currently, in our center imaging of the biliary system by MRCP, followed by ERCP if MRCP is not informative, as well as colonoscopy are part of the evaluation of all children with liver disease associated with autoimmune features.

The adult IAIHG scoring systems for the diagnosis of AIH, as currently formulated, do not allow distinguishing AIH from ASC [7, 76], as they do not include cholangiographic investigations at presentation. The ESPGHAN diagnostic scoring system should be used [2] (Table 66.3).

Treatment

Children with ASC respond usually well to the same immunosuppressive treatment described above for AIH [3, 7, 153] with resolution of liver test abnormalities within a few months in most patients. Response to immunosuppressive drugs is less satisfactory if the disease is long-standing before starting treatment [152, 154].

The medium- to long-term prognosis of ASC is worse than that of AIH because of progression of bile duct disease despite treatment in some 50% of patients, with some 20% of them eventually requiring liver transplantation [3, 7, 153, 160].

Table 66.3 Proposed scoring criteria for the diagnosis of juvenile autoimmune liver disease [2]

Variable	Cut-off	Points	
		AIH	ASC
ANA and/or SMA[a]	≥ 1:20[b]	1	1
	≥ 1:80	2	2
Anti-LKM-1[a] or	≥ 1:10[b]	1	1
	≥ 1:80	2	1
Anti-LC-1	Positive[b]	2	1
Anti-SLA	Positive[b]	2	2
pANNA	Positive	1	2
IgG	> ULN	1	1
	> 1.20 ULN	2	2
Liver histology	Compatible with AIH	1	1
	Typical of AIH	2	2
Absence of viral hepatitis (A,B,E,EBV), NASH, Wilson disease & drug exposure	Yes	2	2
Presence of extrahepatic autoimmunity	Yes	1	1
Family history of autoimmune disease	Yes	1	1
Cholangiography	Normal	2	-2
	Abnormal	-2	2

Score ≥7: probable AIH; ≥8: definite AIH
Score ≥7: probable ASC; ≥8: definite ASC
AIH autoimmune hepatitis, *ASC* autoimmune sclerosing cholangitis, *ANA* antinuclear antibody, *SMA* anti-smooth muscle antibody, *anti-LKM-1* anti-liver kidney microsomal antibody type 1, *anti-LC-1* anti-liver cytosol type 1, *anti-SLA* anti-soluble liver antigen, *IgG* immunoglobulin G, *EBV* Epstein-Barr virus, *NASH* nonalcoholic steatohepatitis, *ULN* upper limit of normal
[a]Antibodies measured by indirect immunofluorescence on a composite rodent substrate (kidney, liver, stomach)
[b]Addition of points achieved for ANA, SMA, anti-LKM-1, anti-LC-1, and anti-SLA autoantibodies cannot exceed a maximum of 2 points
From Mieli-Vergani et al. [2]

Based on a reported beneficial effect in adult primary sclerosing cholangitis, ursodeoxycholic acid (UDCA) is added to immunosuppression in the treatment of ASC, but whether it is helpful in arresting the progression of the bile duct disease remains to be established. In adults with primary sclerosing cholangitis, high-dose UDCA was reported as more beneficial than standard doses [161], but a randomized double-blind controlled study from the Mayo Clinic shows that high-dose (30–40 mg/kg) UDCA has a severe negative effect and was discontinued [162]. It is prudent, therefore, to use doses not higher than 15–20 mg/kg/day.

Reactivation of the liver disease often follows flares of the intestinal disease in sclerosing cholangitis patients with IBD. It is therefore essential to control the bowel pathology to avoid progression of liver disease. It has been suggested that the chronic IBD associated with ASC may represent a distinct nosologic entity, different from classic ulcerative colitis and Crohn's disease, being characterized by right-sided colitis with frequent rectal sparing and small bowel mucosal breaks on capsule enteroscopy [163]. A beneficial effect of oral vancomycin has been reported in a small number of children with sclerosing cholangitis and IBD by the same group [164, 165]. Each report includes 14 pediatric patients, six of whom were described in both series. Vancomycin was given at 50 mg/kg/day (maximum 1500 mg daily), in three divided doses. In the first paper, after 1–2 months of therapy, all 14 patients had a decrease in GGT levels, and nine (64%) normalized them, but five (36%) did not, including the four patients who were cirrhotic before treatment. Bilirubin levels did not improve, and the decrease of GGT levels was dependent on continuation of treatment, as they increased again when oral vancomycin was discontinued. In the second series, nonspecific histological and cholangiographic improvements were reported in all patients after at least 3 months of therapy. Most patients were treated continuously and indefinitely, and none of them developed end-stage liver disease or dominant strictures, but the mean follow-up was less than a year and a half, too short to draw

any conclusion, as only 6% of a large cohort of children with sclerosing cholangitis were shown to progress to end-stage liver disease or dominant strictures within a year and a half of follow-up [166]. Whether vancomycin would have beneficial effects through its antibiotic or immunomodulatory [165] properties remains to be elucidated.

Pathophysiology

It is unclear if the juvenile autoimmune form of sclerosing cholangitis and AIH are two distinct entities or different aspects of the same condition. Akin to AIH, in addition to positivity for ANA and/or SMA, liver-specific autoantibodies, including antibodies to liver-specific lipoprotein, asialoglycoprotein receptor, alcohol dehydrogenase, and soluble liver antigen, can be found in ASC [29, 98, 167]. Susceptibility to ASC in children is conferred by the possession of HLA DRB1*0301 and HLA DRB1*1301, while possession of HLA DRB1*0401 appears to be protective. HLA DRB1*1301 has also been associated with susceptibility to primary sclerosing cholangitis in adults [168]. An association with HLA DRB1*1301 has been reported in children with ANA/SMA-positive AIH in Argentina, but no cholangiographic studies were performed, therefore not excluding the possibility that the cohort comprised also children with ASC [169].

Liver Transplant and Autoimmune Liver Disease

Liver transplantation is indicated in patients with AIH who present with fulminant hepatic failure (with encephalopathy) unresponsive to steroids and in patients with AIH or sclerosing cholangitis who develop end-stage liver disease despite treatment. The latter is more likely when established cirrhosis is present at diagnosis or if there is a long history of liver disease before the start of treatment. Approximately 10% of children with AIH and 20% of those with sclerosing cholangitis require liver transplantation. After transplantation, recurrent AIH has been described in about 20% of cases [170] and recurrent sclerosing cholangitis in 27–67% of transplanted patients [152, 160, 171]. Diagnosis of recurrence is based on biochemical abnormalities, presence of autoantibodies, interface hepatitis on liver histology, steroid dependence, and, for sclerosing cholangitis, presence of cholangiopathy. Recurrence may occur even years after transplantation, and consequently maintenance of steroid-based immunosuppression at a higher dose than that used for patients not transplanted for autoimmune liver disease is recommended. While recurrence of AIH does not usually affect posttransplant outcome, recurrence of ASC leads to re-transplantation in a high proportion of patients [160]. Recurrence of sclerosing cholangitis after transplantation is often associated with uncontrolled IBD [171, 172]. In this context, it is of interest that primary sclerosing cholangitis recurrence in adults with IBD can be prevented by pre-liver transplant colectomy [173–175].

De Novo Autoimmune Hepatitis After Liver Transplantation

In the late 1990s, it was observed that AIH can arise de novo after liver transplantation in children who were transplanted for non-autoimmune liver conditions. Characteristic of this condition is a histological picture of interface hepatitis and multilobular collapse associated with increased IgG levels and positive autoantibodies. These include ANA, SMA, and classical anti-LKM-1 but also atypical anti-LKM-1, staining the renal tubules but not the liver. After the original report [176], de novo AIH following liver transplant has been confirmed by several studies both in adult and pediatric patients [177–181] and has been reported to be more frequent in steroid-free antirejection regimens [182, 183]. Importantly, treatment with prednisolone and azathioprine using the same schedule for classical AIH, concomitant with reduction of the calcineurin inhibitor dose, is highly effective in de novo AIH, leading to excellent medium-term graft and patient survival, though, if allograft dysfunction persists long term, chronic liver damage may ensue leading to re-transplantation [184]. It is of interest that patients with de novo AIH do not respond satisfactorily to the standard antirejection treatment schedule, making it essential to reach an early diagnosis to avoid graft loss. Rapamycin has been reported to be effective in difficult-to-treat patients [185].

Pathophysiology of De Novo AIH After Liver Transplant

Whether the liver damage observed in these patients is a form of rejection or the consequence of an autoimmune injury, possibly triggered by drugs or viral infection, remains to be established.

Several reports have investigated whether the development of de novo AIH is associated with the possession of specific major histocompatibility complex (MHC) antigens either by the recipient or by the donor. In the original report, five of seven children with de novo AIH received livers from donors who were HLA DR3- or DR4-positive [176]. In adults, Heneghan et al. found HLA DR3 or DR4 in either donors or recipients in all cases [186], and Salcedo et al. noted an overrepresentation of DR3 in recipients [187].

There are several non-mutually exclusive explanations as to why autoimmunity and AIH may arise de novo in patients

transplanted for non-autoimmune conditions. Besides autoantigen release from the damaged tissue, molecular mimicry might be involved, where immune responses to external pathogens become directed toward structurally similar self-components [188]. In Salcedo et al.'s series, all patients developed de novo AIH in relation to infection with cytomegalovirus, Epstein-Barr virus, or parvovirus [187]. Viral infections, which are frequent after liver transplant, may lead to autoimmunity also through other mechanisms, including polyclonal stimulation, enhancement and induction of membrane expression of MHC class I and II antigens, and/or interference with immunoregulatory cells [180, 189].

Moreover, calcineurin inhibitors may interfere with the maturation of T-cells and/or with the function of Tregs, with consequent emergence and activation of autoaggressive T-cell clones [189–194]. In the experimental context, calcineurin inhibitor-associated autoimmunity arises in animals treated during the neonatal period or rendered immunocompromised by irradiation, reinforcing the concept that patients treated with immunosuppressants, like prednisolone, after liver transplant, may be predisposed to developing autoimmunity through the influence of calcineurin inhibitors. Cyclosporine blocks activation-induced cell death of effector T-cells and interferes with tolerance induction by co-stimulation blockade [193]. Calcineurin inhibitors also act by reducing IL-2 production [195]. As IL-2 is an indispensable survival and growth factor for Tregs [196], the absence of IL-2 leads to impaired suppressor function [197]. De novo AIH occurs more frequently in patients who develop repeated episodes of acute cellular rejection [183, 186, 198–202], which is also associated with a decreased number of circulating Tregs [203]. Treg impairment, as a consequence of calcineurin inhibitors and/or acute cellular rejection, may contribute to the perpetuation of an autoreactive immune response and to the development of de novo AIH.

Since children have an immature immune system—therefore an active thymus and an immature T-cell-receptor repertoire—and are exposed to numerous primary infections, they may be more vulnerable to autoimmunity under calcineurin inhibitors' influence [129, 198]. This would account for the reportedly higher incidence of de novo AIH in pediatric age compared to adults.

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Liver-Based Inherited Metabolic Disorders

67

Roshni Vara

Introduction

The liver is the central metabolic organ in the body, and there are now more than 400 monogenic disorders described involving the liver [1]. Hepatocytes play a key role in several biochemical pathways including protein synthesis, bile production and metabolism of proteins, fats and carbohydrates. Disorders of mitochondrial, lysosomal and peroxisomal function can also present with liver manifestations. Inherited metabolic diseases (IMD) account for approximately one third of paediatric patients presenting with hepatomegaly, cholestasis, acute liver failure and chronic liver disease [2]. IMDs now account for 15–20% of all paediatric liver transplants in some centres.

Clinical manifestations of liver-based IMDs can be detected in the neonate, infancy, childhood and adulthood. Early diagnosis and intervention is vital and can impact mortality and morbidity in some of these disorders.

The majority of these disorders are inherited in an autosomal recessive manner, and hence genetic counselling for affected families is important.

Clinical Presentation

Liver-based metabolic disorders can present at any age but tend to be more common in the neonatal period and infancy. However, mitochondrial disorders, for example, can present anywhere from birth to adulthood. Clinical manifestations include hepatomegaly, cholestasis, acute liver failure, hepatic steatosis and chronic liver disease (Table 67.1). There is often overlap of more than one clinical manifestation in inherited metabolic diseases, and hence the table is a guide.

Clinical History and Examination

IMDs are rare and heterogeneous with often non-specific presentations, which can overlap with other liver diseases. Diagnostic clues can be sought from the history and examination to indicate an IMD. Many disorders are inherited in an autosomal recessive manner, and their information regarding consanguinity or other affected individuals should be sought. There may be a positive family history, previous miscarriages, sudden infant deaths and early neonatal deaths with or without a previous diagnosis. The mode of inheritance should be considered carefully when taking a family history. Predominantly affected males may indicate an X-linked inheritance pattern, and a matrilineal inheritance pattern may suggest a mitochondrial DNA point mutation. In some babies with fatty acid oxidation defects, there may be a history of acute fatty liver of pregnancy (AFLP) or maternal HELLP syndrome (haemolysis, elevated liver enzymes and low platelets); the association has been previously reported in association with long-chain fatty acid oxidation defects [3, 4].

There is often a symptom-free period in intoxication-type IMDs, and neonates tend to present in the first few days of life. There is often a trigger in the form of fasting, intercurrent infections or surgery. In the past medical history, there may be specific food aversions, e.g. protein in urea cycle defects and specific sugars in hereditary fructose intolerance. There may be a history of developmental delay or intermittent episodes of clinical manifestations. IMDs affecting the liver can also be multisystemic, and hence it is important to identify other clinical signs and symptoms.

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Table 67.1 Clinical presentations of metabolic liver disease

Clinical presentation	Differential diagnoses	Associated features
Hepatomegaly		
	GSD I a/b, III, IV and IX	Hypoglycaemia Ketosis Lactic acidosis, hyperuricaemia, hyperlipidaemia (GSD I) Neutropenia (GSD Ib) Short stature
	Fanconi–Bickel syndrome	Hypoglycaemia Renal tubulopathy
	Fructose-1,6-bisphosphatase deficiency	Lactic acidosis Hypoglycaemia
	Lysinuric protein intolerance	Elevated ferritin
	Cholesterol ester storage disorder	Failure to thrive Splenomegaly
	Lysosomal storage disorders	+ / – splenomegaly Coarse facial features
	Niemann–Pick disease	+ / – splenomegaly Cholestasis (NPC)
Cholestasis		
	Galactosaemia	Coagulopathy
	Citrin deficiency	Failure to thrive
	Congenital disorders of glycosylation	Dysmorphic features
	Bile acid synthesis disorders	
	Peroxisomal disorders	Dysmorphic features CNS involvement
	α1-antitrypsin deficiency	
	Progressive familial intrahepatic cholestasis	Low GGT (PFIC1 and BSEP deficiency)
Acute liver failure		
	Tyrosinaemia type 1	Splenomegaly/chronic liver disease Elevated AFP
	Hereditary fructose intolerance	Specific food aversion
	Fatty acid oxidation defects	Elevated CK, hypoketotic hypoglycaemia
	Mitochondrial disorders	Multi-organ involvement
	Urea cycle disorders	Hyperammonaemia Encephalopathy
	Organic acidaemias	Metabolic acidosis Hyperammonaemia
Hepatic steatosis		
	Cholesterol ester storage disorder	Elevated cholesterol/LDL/low HDL
	Glycerol-3-phosphate dehydrogenase 1 deficiency (GPD1)	Hypertriglyceridaemia Hepatomegaly
	Abetalipoproteinaemia	Fat-soluble vitamin malabsorption Failure to thrive
Chronic liver disease/ cirrhosis		
	GSD IV	Hepatomegaly
	Transaldolase deficiency	Dysmorphic features
	Argininosuccinate lyase deficiency	Hyperammonaemia

IMDs do not often have specific findings on clinical examination; however, some manifest with dysmorphic features such as peroxisomal disorders (large anterior fontanelle and forehead, hypotonia), congenital disorders of glycosylation (abnormal fat distribution, inverted nipples) and lysosomal storage disorders (coarse facial features). A full neurological examination is important in establishing any features of central nervous system involvement. Formal ophthalmological examination can reveal classical features in some IMDs such as 'oil drop' cataracts in untreated galactosaemia and cherry red spots in Niemann–Pick disease, and pigmentary retinopathy is a feature of long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency and some mitochondrial disorders. Eye movement disorders are a feature of Niemann–Pick C disease (supranuclear gaze palsy), Gaucher disease (horizontal gaze palsy) and mitochondrial disorders (nystagmus, external ophthalmoplegia).

Investigations

Investigations can be divided into first and second line, and initial biochemical markers can indicate a defect in a specific pathway, which can be elucidated with further targeted testing (Table 67.2).

Baseline metabolic investigations should include:

- Blood sugar.
- Blood or urine ketones.
- Blood gas and lactate.
- Plasma ammonia.

Table 67.2 Summarises metabolic investigations with specific disorders and aspects of hepatic presentation

Investigation	Condition	Metabolite/finding	Hepatic presentation
Plasma amino acids	Tyrosinaemia type 1 Urea cycle defects Citrin deficiency Lysinuric protein intolerance	↑ tyrosine/methionine ↑ glutamine ↓ arginine (OTC) ↑ citrulline (citrullinaemia) ↑ arginine (arginase deficiency) ↑ citrulline ↑ threonine/serine ratio ↓ lysine, ornithine, arginine (plasma)	ALF, CLD ALF, E C, ALF, HS H, E, HS
Urine organic acids	Organic acidaemia	↑ specific organic acids	ALF, E
	Tyrosinaemia type 1	↑ succinylacetone	ALF, CLD
	Argininosuccinic aciduria	↑ argininosuccinic acid	ALF, CLD, E
Acylcarnitines	Fatty acid oxidation defects Organic acidaemias	↑ specific acylcarnitines ↑ specific acylcarnitines Often low free carnitine	ALF, HS, H, E
Lactate	Mitochondrial disorders GSD 1 a/b GSD III	↑ ↑ with fasting ↑ post prandially	ALF, CLD, HS H, HS H
Creatine kinase	Fatty acid oxidation defects GSD III	↑ ↑	
Lipid profile	Cholesterol ester storage disorder GSD	↑ cholesterol/LDL ↓ HDL ↑ triglycerides	H, CLD, HS H, HS
Urate	GSD 1 a/b	↑	H
α-Fetoprotein	Tyrosinaemia type 1	↑↑	ALF, CLD
Bile acids	Bile acid synthesis defects	Specific abnormal bile acids	C, CLD
Very-long-chain fatty acids (VLCFA)	Peroxisomal disorders	↑	C, ALF, CLD
Transferrin glycoforms	Congenital disorders of glycosylation Hereditary fructosaemia/galactosaemia	Type 1 or 2 pattern Type 1 pattern (untreated)	C, CLD, ALF, H C, ALF, H, HS
α1-antitrypsin phenotype	α1-antitrypsin deficiency	Pi M/Z/S	C, CLD
Galactose-1-phosphate uridyl transferase	Galactosaemia	↓↓ affected | heterozygote	ALF, C
Oxysterols	Niemann–pick type C	↑	C, ALF
Polyols (urine or plasma)	Transaldolase deficiency	↑ sedoheptulose, ribitol, erythritol and arabitol	CLD, H
Vacuolated lymphocytes	Lysosomal storage disorders	Storage cells	H
Urine glycosaminoglycans	Lysosomal storage disorders	Specific patterns	H
White cell enzymology	Lysosomal storage disorders	Specific deficiencies	H
Bone marrow aspirate	Lysosomal storage disorders	Storage cells	H
Skin biopsy	Lysosomal storage disorders Mitochondrial disorders FAODs	Filipin staining abnormal (NPC) Respiratory chain complex (RCC) activity Fatty acid oxidation studies	H ALF, CLD ALF, H
Muscle biopsy	Mitochondrial disorders	RCC activity Abnormal histology	ALF, CLD
Abdominal X-ray	Cholesterol ester storage disease (infantile form)	Adrenal calcification	H, HS

C cholestasis, *H* hepatomegaly, *ALF* acute liver failure, *CLD* chronic liver disease, *E* encephalopathy, *HS* hepatic steatosis

- Plasma amino acids.
- Urine organic acids.
- Acylcarnitines (dried blood spot or plasma).
- Creatine kinase.
- Lipid profile.
- DNA store (EDTA sample).

Urine amino acids are rarely useful in a baseline screen unless a specific transporter defect is suspected, e.g. lysinuric protein intolerance. Urine reducing substances are also rarely used in recent years due to lack of sensitivity and specificity, and if galactosaemia is suspected clinically, formal enzymology should be sent.

Formal investigation of multi-organ involvement may be required and may include renal, cardiac and central nervous system assessment with glomerular filtration rate, renal tubulopathy screen, electrocardiogram, echocardiography, magnetic resonance imaging (MRI) brain, electroencephalogram (EEG) and muscle biopsy.

Liver imaging may be helpful in elucidating the presence of fatty liver or evidence of chronic liver disease. Liver biopsy can often indicate an IMD with the presence of micro- or macrovesicular steatosis, glycogenated hepatocytes and the presence of storage material.

Molecular diagnosis for IMDs is required for confirmation and can be used for prenatal testing or cascade screening. The use of gene panels with next-generation sequencing has revolutionised the diagnosis of IMDs presenting with liver disease and the ability to provide targeted therapy and early intervention [5]. The use of rapid diagnosis, for example, in mitochondrial disorders presenting with neonatal liver failure can avoid liver transplantation in cases with an extremely poor prognosis. In more recent years, whole exome (WES) and whole genome sequencing (WGS) is increasingly used, particularly in complex and undiagnosed cases [6].

Newborn screening programmes identify some IMDs and allow early therapeutic intervention. In the UK, screening is limited to six IMDs: medium-chain acyl CoA dehydrogenase deficiency (MCADD), phenylketonuria (PKU), isovaleric acidaemia (IVA), maple syrup urine disease (MSUD), glutaric aciduria type 1 (GA1) and homocystinuria (HCU). MCADD can present with hypoketotic hypoglycaemia, hepatomegaly and acute liver failure if left undetected. The other disorders do not tend to have hepatic involvement. Galactosaemia can occasionally be detected incidentally when phenylalanine and tyrosine are found to be elevated as a consequence of screening for PKU in which the phenylalanine is elevated in relation to tyrosine; however, with liver dysfunction of any cause, both can be elevated. Worldwide newborn screening programmes are variable and include conditions such as tyrosinaemia type 1, galactosaemia and urea cycle defects [7].

Disorders of Carbohydrate Metabolism

Galactosaemia

Classical galactosaemia is an autosomal recessive disorder due to deficiency of galactose-1-phosphate uridyl transferase (GALIPUT) and typically presents in the first week of life when milk feeds are introduced. The introduction of galactose in the form of lactose leads to the accumulation of toxic metabolites including galactose-1-phosphate and galactitol (Fig. 67.1).

The incidence in the UK is approximately 1 in 45,000 live births, and the *GALT* gene is located on chromosome 9 and inherited in a recessive fashion. There is a common mutation Q188R identified in 72% of cases with classical galactosaemia. Genotype–phenotype correlations are unclear; however, the Duarte variant (N314D) has 50% residual enzyme activity and is a benign variant [8]. The neonate typically presents with poor feeding, failure to thrive, cholestasis, hypoglycaemia, liver dysfunction or acute liver failure with coagulopathy as a prominent feature. Ophthalmological examination may reveal the classical 'oil drop' cataracts, which resolves with treatment. Neonatal sepsis typically with *Escherichia coli* can also accompany the hepatic presentation, and hence the threshold for antibiotic therapy should be low. Raised intracranial pressure due to cerebral oedema has been reported [9]. A more insidious presentation with symptoms beyond the first few weeks of life can occur with failure to thrive, cataracts, renal tubulopathy and very rarely developmental delay. Diagnosis is made by measurement of GALIPUT activity in erythrocytes with a clear distinction between affected (absent or barely detectable), heterozygote carriers and unaffected cases. Care must be taken in neonates who have received a red cell transfusion within 6 weeks of testing as this may lead to false-negative results. In this circumstance, parental enzyme testing is recommended, as there is a well-defined heterozygote range. Galactose-1-phosphate levels can be measured in some laboratories and will be elevated in untreated cases.

Galactose epimerase deficiency is due to uridine diphosphate-galactose-4′-epimerase (GALE) deficiency and can present in a similar fashion to classical galactosaemia. It should be considered when there is a strong clinical suspicion of galactosaemia and the GALIPUT activity is normal. Diagnosis confirmation is made genetically. Galactokinase deficiency is another disorder of galactose metabolism; however, the phenotype is restricted to ophthalmic manifestations without liver involvement.

Prompt exclusion of lactose from the diet results in resolution of clinical manifestations; hence, if the diagnosis is suspected, a lactose-free formula should be used until the diagnosis is excluded. The use of a casein-based protein

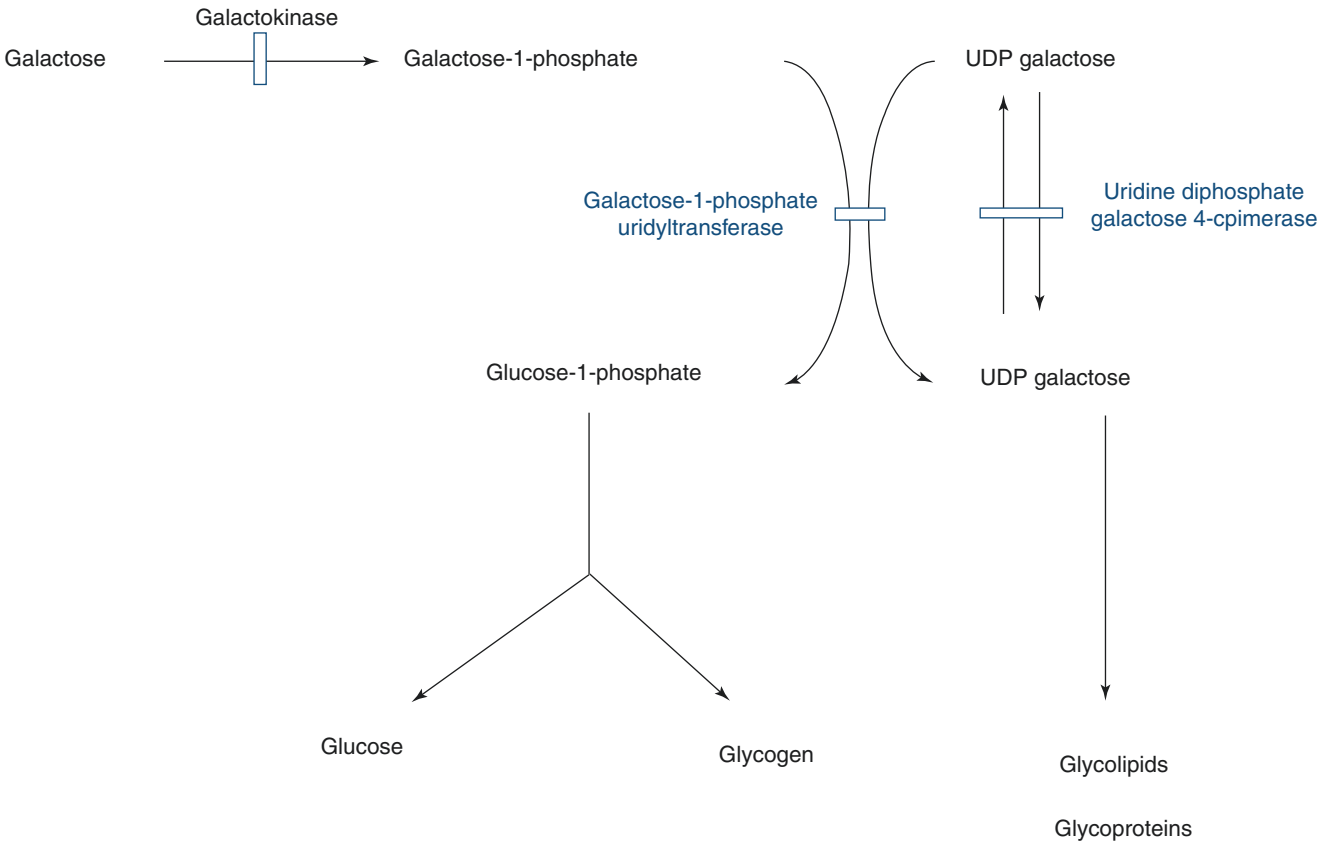

Fig. 67.1 Metabolism of galactose and associated disorders. *UDP* uridine diphosphate

hydrolysate formula (e.g. Pregestimil) is often used when cholestasis and liver dysfunction is prominent with conversion to a soya-based formula once this has resolved. If soya-based formula is not tolerated, an elemental formula (e.g. Neocate) can be used. A lifelong dietary galactose restriction is recommended, and practices are variable worldwide with regard to severity of restriction and necessity in later life [10, 11]. Calcium intake and vitamin D sufficiency need to be regularly assessed at outpatient visits.

Despite treatment, long-term complications of classical galactosaemia are not abolished and include speech and language delay, learning difficulties, neurocognitive issues, movement disorders, osteoporosis and primary ovarian failure in females [12, 13]. In view of these complications, despite even presymptomatic treatment, newborn screening for galactosaemia remains controversial [14].

Hereditary Fructose Intolerance (HFI)

HFI is an autosomal recessive disorder due to deficiency of aldolase B. The key to diagnosis is establishing a clear history of fructose, sucrose or sorbitol intake prior to the onset of symptoms. Fructose is a monosaccharide that is found in many food sources, notably sucrose (glucose–fructose disaccharide), fruits, vegetables and honey. Sorbitol is an artificial sweetener that is metabolised to fructose. The classical presentation is that of a healthy milk-fed infant that develops symptoms when it is first exposed to fructose upon weaning. Occasionally, infants may receive inadvertent fructose prior to weaning as a soother in some cultures. Gastrointestinal upset progresses to persistent vomiting, sweating, lethargy and coma if intake continues. These symptoms are accompanied by liver failure and evidence of renal proximal tubulopathy. Hypoglycaemia and hyperlactatemia are common but can be masked by glucose administration. There is a considerable spectrum of disease severity; infants can present with a chronic course of failure to thrive, hepatomegaly and liver impairment. School-age children may present with avoidance of sweet foods and hepatomegaly, with dentition that is unusually free of caries. Patients may develop self-selected food aversion to avoid sources of fructose that allow them to remain quite well.

Biochemical associations include lactic acidosis, hyperuricaemia, marked liver dysfunction, often without jaundice and renal tubulopathy. The enzyme deficiency results in accumulation of fructose-1-phosphate, an inhibitor of both glycogenolysis and gluconeogenesis that depletes inorganic phosphate, thus restricting adenosine triphosphate (ATP) production. Cellular depletion of ATP is postulated to be a significant mechanism of hepatocellular toxicity [15].

There is no rapid test that can exclude HFI. If it is suspected clinically, then fructose should be immediately excluded. A rapid correction of biochemistry and liver function can be expected over a number of days to weeks. Transferring glycoforms can show a type 1 pattern of glycosylation in the untreated state. Hepatomegaly and transaminasemia may take several months to resolve. Direct enzyme assay is restricted to liver tissue, and liver biopsy may be contraindicated in the acute phase due to coagulopathy. Genotyping of the *ALDOB* gene can often provide a definitive diagnosis; however, if pathogenic variants are not detected, enzymology may need to be performed if clinical suspicion remains high.

Treatment requires specialist dietetic input with a lifelong restriction of fructose, sucrose and sorbitol from the diet and can be a challenge due to its restrictive nature. In addition, medicines and formulas in which fructose/sucrose may not be listed as a primary component need to be avoided. During hospitalisations, special caution is advised to avoid the use of fructose-containing intravenous fluids. Given that reduced fruit and vegetable intake is a dietary requirement, daily supplementation with a 'sugar-free' multivitamin is recommended to prevent micronutrient deficiencies, specifically water-soluble vitamins, vitamin C and folate. No formal guidelines for surveillance exist. Once the diagnosis of HFI has been made, annual assessment of liver function, renal function and growth is recommended, particularly if compliance with the fructose/sucrose/sorbitol-restricted diet is not absolute. Long-term outcomes are good if treatment is adhered to [16].

Glycogen Storage Disorders

Glycogen is a branched polysaccharide structure consisting of glucose units and is the main storage form of glucose primarily in the liver and muscle tissue. During times of need, glycogen is rapidly broken down to form glucose; in the muscle, this provides an immediate source of energy during the first 30 minutes of exercise [17]. Hepatic glycogen is broken down to release free glucose into the bloodstream and is crucial for maintaining blood glucose levels to support the needs of other tissues. Glycogen synthesis and degradation are highly regulated multistep processes involving distinct sets of enzymatic steps (Fig. 67.2).

Glycogen storage disorders result from abnormalities in the function of the enzymes that control the synthesis, regulation and degradation of glycogen.

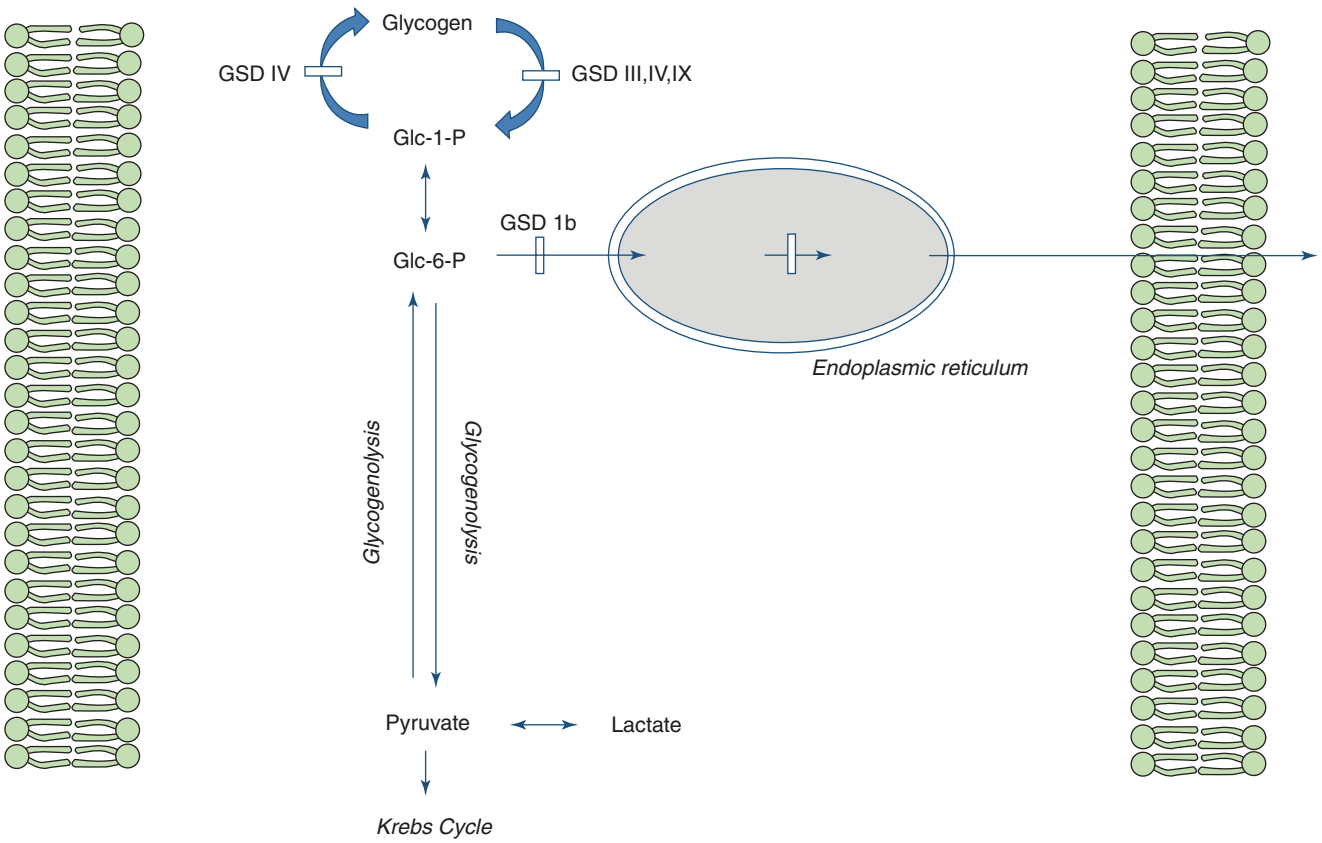

Fig. 67.2 Glycogen metabolism in the hepatocyte. *GSD* glycogen storage disorders, *Glc* glucose, *Glc-6-P* glucose-6-phosphate, *Glc-1-P* glucose-1-phosphate

Cumulatively, the incidence of GSDs is rare (<1:20,000), and all are inherited in an autosomal recessive fashion, with the exception of GSD type IX and Danon disease (X-linked recessive). Although abnormal glycogen storage is a hallmark, there is a large spectrum of phenotypes associated with these disorders, with the age of onset ranging from in utero to adulthood [18]. GSDs are primarily classified according to the affected enzyme, and the focus will be hepatic GSDs (type 1, III, IV, VI and IX) for the purposes of this chapter (Table 67.3).

Common clinical manifestations of GSD are significant hepatomegaly, occasionally splenomegaly, hypoglycaemia (which may be masked by frequent feeding) and short stature. The suspicion of GSD is often raised following investigation of an incidental finding of hepatomegaly or episodes of hypoglycaemia.

GSD 0 is due to glycogen synthase deficiency and causes a block in glycogen synthesis. It typically presents with ketotic hypoglycaemia and short fasting tolerance in the absence of hepatomegaly. There is often postprandial hyperglycaemia and hyperlactatemia [19].

GSD type 1 is the most severe form of GSD and often presents in the first 6 months of life. The abnormal fat distribution gives a 'doll-like' facial appearance, and splenomegaly and nephromegaly may be present. There are two subtypes: 1a and 1b. Glucose-6-phosphatase deficiency or GSD 1a accounts for 80%. G6Pase catalyses the conversion of G6P to glucose and inorganic phosphate. G6Pase is anchored onto the endoplasmic reticulum and requires the G6P transporter (G6PT) to bring G6P into the ER lumen before it is hydrolysed [20]. A defect in this translocase activity is known as GSD 1b. The primary metabolic abnormality of both subtypes is fasting hypoglycaemia because glucose cannot be formed from G6P that is produced from either via gluconeogenesis or glycogenolysis. The elevated G6P leads to associated biochemical abnormalities, i.e. lactic acidosis, hyperuricaemia and hyperlipidaemia. Neutropenia, recurrent infection due to abnormal neutrophil function and inflammatory bowel disease are unique features of GSD 1b. More recently, empagliflozin, a SGLT2 inhibitor, has been shown to improve neutropenia and neutrophil dysfunction in GSD 1b patients [21].

Dietary intervention is the cornerstone of management with regular carbohydrate intake to maintain good metabolic control and avoid long-term complications of disease. Traditional approaches in infants utilise continuous overnight feeding regimens to maintain normoglycaemia and reduction of lactate. In addition, the use of uncooked cornstarch aims to maintain normoglycaemia during the day and night as required. Guidelines for the dietary management of GSD 1 are well published [22, 23]. If treatment is not optimal, long-term complications of GSD 1 can occur, which include osteoporosis, hyperuricaemia, nephrolithiasis, anaemia and liver adenomas [24, 25].

GSD type III is caused by a deficiency of the glycogen debrancher enzyme that results in the accumulation of abnormal glycogen. It may be clinically indistinguishable from type I, but fasting tolerance/tendency for hypoglycaemia is typically not as severe as type I, and nephromegaly is not a feature. Lactate characteristically increases postprandially, and creatine kinase is commonly raised. The mixed hepatic and muscle form is the most common (type IIIa). Muscle weakness is not common in childhood and becomes more prominent in adults, although motor milestones may be delayed and activity can be impaired. Cramps at night are common. Long-term outcome in the purely hepatic form (type IIIb) is good. Hepatic adenoma is rare, and reports of cirrhosis and progression to liver failure are infrequent. In type IIIa complications include progressive myopathy and cardiomyopathy although the latter is rarely clinically symptomatic [26, 27].

GSD type IV is extremely rare and is due to deficiency of the branching enzyme and mutations is *GBE1* gene, resulting in the formation of abnormal glycogen known as amylopectin or polyglucosan. The clinical manifestations range from a severe neonatal neuromuscular form (often fatal), a progressive hepatic form and nonprogressive hepatic form. Cardiomyopathy can be present. Children tend to present with hepatomegaly, evidence of chronic liver disease and classical findings on liver biopsy. The majority have an

Table 67.3 Nomenclature of hepatic glycogen storage disorders

Type	Enzyme	Gene	Inheritance
Ia (von Gierke)	Glucose-6-phosphatase	*G6PC*	Recessive
Ib	Glucose-6-phosphate translocase	*G6PT1/SLC37A4*	Recessive
IIIa (Cori/Forbes)	Glycogen debrancher (liver and muscle)	*AGL*	Recessive
IIIb (Cori/Forbes)	Glycogen debrancher (liver)	*AGL*	Recessive
IV (Andersen)	Glycogen branching enzyme	*GBE1*	Recessive
VI (hers)	Liver glycogen phosphorylase	*PYGL*	Recessive
IXa (XLG)	α-Subunit phosphorylase b kinase (liver)	*PHKA2*	X-linked
IXb	β-Subunit phosphorylase b kinase (liver/muscle)	*PHKB*	Recessive
IXc	γ-Subunit phosphorylase b kinase (liver)	*PHKG2*	X-linked
0a	Liver glycogen synthase	*GYS2*	Recessive

absence of fasting hypoglycaemia. Recently, the clinical spectrum of this disorder has widened considerably [28]. Liver transplantation is the only treatment option for those with the progressive hepatic form who can go on to develop decompensated liver disease. Regular liver and cardiac surveillance is recommended [29].

Phosphorylase (GSD type VI) and its activator phosphorylase b kinase (GSD type IX) are required for the removal of glycosyl molecules from the straight chains of glycogen. GSD VI presents with hepatomegaly and a tendency to ketotic hypoglycaemia in early childhood. Growth failure may be marked, but catch-up growth occurs with treatment, and normal adult height is achieved. The disorder may be so mild that it remains undiagnosed. Long-term outlook is generally excellent, although hepatic adenomas have rarely been described. GSD type IX has a number of subtypes including three that have a hepatic component. Type IXa is by far the most common and has a relatively mild isolated hepatic phenotype similar to type VI. Type IXb is very rare and has a mild mixed liver/muscle phenotype. Type IXc has more severe phenotype often associated with significant hypoglycaemia and progression to cirrhosis [30–33].

Confirmation of a suspected diagnosis of GSD requires assessment of biochemical characteristics (Table 67.4) and ultimately confirmation with molecular diagnosis. The use of enzyme assays for diagnosis has more recently been superseded by genetic panel testing [34]. Liver biopsy may often be performed as part of investigation for hepatomegaly and glycogenated hepatocytes, and occasionally steatosis may be present. Periodic acid–Schiff (PAS) staining reveals cytoplasmic glycogen deposition.

Treatment regimens are tailored for individual patients and necessitate correction of metabolic abnormalities and maintenance of normal growth and development. Regular assessments are required to monitor for complications of individual GSDs and routine follow-up in outpatient clinics with specialist dietary guidance [35].

Liver transplantation is occasionally an option in GSDs with progressive liver disease or hepatocellular carcinoma and in those whom metabolic control is not maintained with medical therapy alone. Liver transplantation for GSD 1b may not correct the neutropenia and inflammatory bowel disease complications and is very rarely required in types VI and IX. The consideration for liver transplantation should be made as part of a multidisciplinary team and on an individual case basis [36, 37].

Fanconi–Bickel Syndrome

The Fanconi–Bickel syndrome, previously classified as type GSD XI, presents with marked hepatomegaly secondary to glycogen storage and fasting hypoglycaemia. Other features include postprandial hyperglycaemia, hypergalactosaemia and renal Fanconi syndrome. The primary defect is a deficiency of the glucose 2 (Glut2) transporter, which is important in the uptake and release of glucose from the liver. Glut 2 is also expressed in the pancreas, gut and kidney. Defects impair glucose sensing within islet β-cells compounding hyperglycaemia in the fed state, due to decreased liver uptake, by blunting the insulin response. Hypoglycaemia, in the fasting state, is secondary to impaired glucose release from the liver. Increased intrahepatic glucose inhibits glycogen degradation facilitating storage, and glycogen deposition may be noted on liver biopsy. Diagnosis relies on recognition of abnormal glucose homeostasis and renal tubulopathy, while transaminitis, hyperlipidaemia and hyperuricaemia may be present. Treatment focuses on dietary support of glucose homeostasis with regular feed and also active replacement of renal losses [38].

Fructose-1,6-Bisphosphatase Deficiency

Fructose-1,6-bisphosphatase (FDP) deficiency is a defect in gluconeogenesis, the pathway that generates endogenous glucose and is a crucial mechanism to maintain glucose homeostasis when dietary glucose is depleted. It is also required for the metabolism of exogenous fructose. The classical features of FDP are hypoglycaemia, lactic acidosis and prominent hepatomegaly. Approximately 50% of cases present within the first 4 days of life and the majority by 6 months of age. Acute acidosis causes hyperventilation and irritability that can progress to coma, apnoea and cardiac arrest. Acute

Table 67.4 Biochemical characteristics of hepatic GSDs

GSD	Hypoglycaemia	Lactate	Ketones	Triglycerides	Cholesterol	CK	ALT/AST
I	++	+	–	++	+	–	++
III	++	+ [a]	+	+	+	+	++
IV	–	–	–	–	–	+ / –	+
VI	+ / ++	+ [a]	+ / ++	+	– / +	–	+
IX	+ / ++	+ [a]	+ /++	+	– / +	–	+
0	+ / ++	+ [a]	+	–	–	–	– /+

[a] Postprandial

hepatomegaly is commonly found, with elevated lactate and ketosis that can be evident (an unusual finding in neonates). Diagnosis relies on clinical suspicion that is confirmed by enzyme analysis of FDP in leucocytes (or liver) or genetic analysis of the *FBP1* gene. It is an autosomal recessive condition, and a family history of neonatal death/acidosis is not uncommon.

Treatment of the acute presentation involves vigorous supplementation of glucose to inhibit gluconeogenesis. The acidosis and hepatomegaly generally respond promptly to this therapy. The acidosis may be severe enough to warrant the use of bicarbonate to correct acid–base balance. Mild hepatomegaly may persist during infancy but is not associated with signs of liver disease. Long-term treatment involves strict avoidance of fasting and adequate supply of carbohydrate during intercurrent illness. Dietary sources containing significant amounts of fructose (including sucrose/sorbitol) should be avoided in the acute situation, but fructose does not have to be rigorously excluded as in HFI. Prognosis is excellent as long as metabolic decompensation is avoided and managed correctly [39].

Transaldolase Deficiency

Transaldolase deficiency is a very rare single enzyme defect of the pentose phosphate pathway. There is a wide phenotype, but some cases described present uniformly in the neonatal period with hepatosplenomegaly and liver impairment. Associated features included dysmorphism (including cutis laxa), cardiac anomalies, oedema, renal abnormalities, haemolytic anaemia and thrombocytopenia. Diagnosis relies on clinical suspicion followed by analysis of urine polyols. Confirmation of diagnosis can be achieved by enzyme assay in lymphocytes, erythrocytes, fibroblasts or liver tissue and more recently by rapidly available analysis of the *TALDO1* gene. Prognosis may be poor with developmental delay and commonly death within the first year of life associated with liver failure. More recently, further cases have been reported and aid family counselling and management of these patients [40, 41].

Congenital Disorders of Glycosylation (CDG)

The CDs are a rare and large group of heterogeneous disorders, which often present a diagnostic challenge. Since their first clinical description in 1980, more than 100 different types of CDG have been described. Glycosylation is the addition and modification of complex carbohydrate molecules (glycans) to proteins and lipids. The majority of extracellular, membrane-bound and some intracellular proteins are glycosylated, and the glycans perform a wide range of functions from structural roles to cell–cell signalling. The process of glycosylation is a highly complex intracellular mechanism, the understanding of which is continuously being updated as new disorders are described [42]. There are mainly two categories of glycosylation: N-glycosylation and O-glycosylation. N-glycans are linked to the amide group of asparagine, while O-glycans are linked to the hydroxyl group of serine or threonine.

The liver is the major site of glycosylation in the body, and disturbances in glycosylation can affect liver development, structure and function. The clinical phenotype of CDGs is extremely broad; often, multisystem and hepatic involvement is described in several types [43, 44].

MPI-CDG results from deficiency of mannose phosphate isomerase and manifests in the first months of life with liver dysfunction and hepatic fibrosis in the majority of patients without neurological involvement. Oral mannose has been shown to be beneficial for the liver disease.

PMM2-CDG (phosphor-aminomutase 2 deficiency) typically presents in the neonatal period with dysmorphism, abnormal fat distribution, inverted nipples, abnormal coagulation factors, hyperinsulinaemic hypoglycaemia and hepatomegaly. Liver histology can show steatosis and fibrosis with myelin-like lysosomal inclusions within hepatocytes on electron microscopy.

Phosphomannose isomerase deficiency (MPI-CDG) presents primarily with a hepatic and gastrointestinal phenotype and has an effective treatment with mannose supplementation. Typical presentation is within the first year of life with vomiting, abdominal distension, protein-losing enteropathy, GI bleeding and liver dysfunction.

TMEM199-CDG is due to a defect in the transmembrane protein 199 and is an example of a purely hepatic CDG. The few reported patients show mild, subclinical hepatic phenotype without liver failure or cholestasis but with elevated LDL-cholesterol and alkaline phosphatase in all patients. There is mild liver copper accumulation and ceruloplasmin are low, and there is some resemblance to Wilson's disease. On transferrin isoelectric focusing (IEF), there is a type 2 pattern.

Diagnostic screening relies on analysis of glycosylated proteins, and the most widely used is transferrin IEF. An abnormal pattern (either type 1 or 2) often precedes further testing with genetic analysis with next-generation sequencing or whole exome sequencing. It should be noted that a type 1 pattern can be seen in untreated galactosaemia or HFI. If the pattern is normal and the clinical suspicion remains high, genetic testing should be pursued in cases with undiagnosed liver disease and other organ involvement. The liver is involved in approximately 22% of CDGs and is highly variable and often not in isolation.

Disorders of Protein Metabolism

Tyrosinaemia Type 1

Tyrosinaemia is an autosomal recessive disorder with an The metabolic defect responsible for tyrosinaemia type 1, fumarylacetoacetase deficiency, arises late in the catabolic pathway of tyrosine (Fig. 67.3). The immediate precursors to the block fumarylacetoacetate and maleylacetoacetate are cytotoxic to the liver and kidney, with their reduced derivatives including succinylacetone [45]. The early-onset form presents with liver failure, coagulopathy, jaundice and ascites in the first 6 months of life. Hypoglycaemia may result from liver dysfunction or hyperinsulinism secondary to islet cell hyperplasia and may require treatment with diazoxide in the acute presentation [46]. Attenuated forms may present with hypophosphatemic rickets secondary to a renal Fanconi syndrome and evidence of chronic liver disease. Occasionally, children can present with neurological crises, which resemble acute porphyria with abdominal pain, hypertension, peripheral neuropathy and muscle weakness.

The management of tyrosinaemia type 1 has been revolutionised by nitisinone, originally developed as a herbicide, with rapid resolution of liver and renal dysfunction [47]. Prior to 1992, liver transplantation was the only treatment option. Treatment with a phenylalanine- and tyrosine-restricted diet is required in addition to home dried blood spot monitoring of tyrosine and phenylalanine levels; the risk of hepatocellular carcinoma is markedly reduced in patients treated early (<6 months). α-Fetoprotein monitoring in clinics should therefore continue with annual imaging of the liver. Current management combines oral nitisinone at 1 mg/kg/day with a tyrosine- and phenylalanine-restricted diet supplemented with a tailored amino acid supplement. Target tyrosine levels are below 400 μmol/l and nitisinone levels 20–40 μM [48]. Non-adherence to nitisinone can result in acute porphyric-like crises with intermittent abdominal pain, muscle weakness and even respiratory failure that could mimic the progressive weakness in Guillain–Barre syndrome. Interruption of treatment leads to inhibition of porphobilinogen synthase by excess succinylacetone.

Indications for liver transplant include failure of response no significant improvement in prothrombin time within 14 days of commencing treatment or a bilirubin >100 mMol/l despite increasing dose to 2 mg/kg/day and suspicion of hepatocellular carcinoma (development of liver nodule or persistently elevated or rising α-fetoprotein) [49]. Patients presenting later may do less well in the longer term as the liver has been subjected to chronic exposure to the toxic metabolites and therefore is more likely to have cirrhotic change. Concerns remain as to the cognitive outcomes of treated patients, and further work is needed to delineate whether this is due to the condition or a long-term nitisinone effect [50].

Urea Cycle Disorders

The urea cycle converts waste nitrogen to urea for excretion via the kidney and is the site of synthesis of the nonessential amino acid arginine. Enzyme defects within the pathway result in hyperammonaemia and encephalopathy (Fig. 67.4). Inheritance is autosomal recessive with the exception of ornithine transcarbamylase (OTC) deficiency, which is

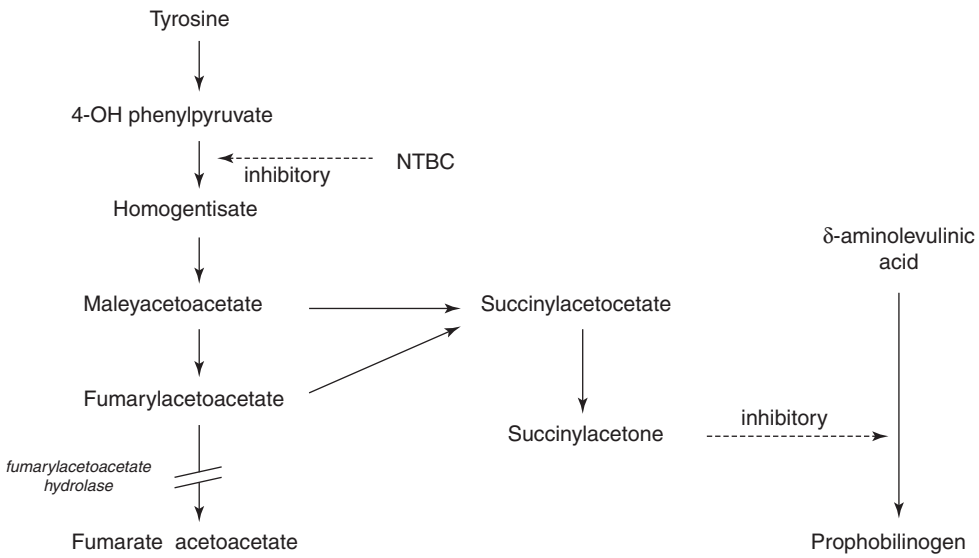

Fig. 67.3 Diagram showing biochemical pathway of tyrosine catabolism, defective step causing tyrosinaemia type I and the action of the substrate reduction therapy nitisinone

NTBC: (2-(2-intro-4-trifluoromethylbenzol)-1,3-cyclohexendiome

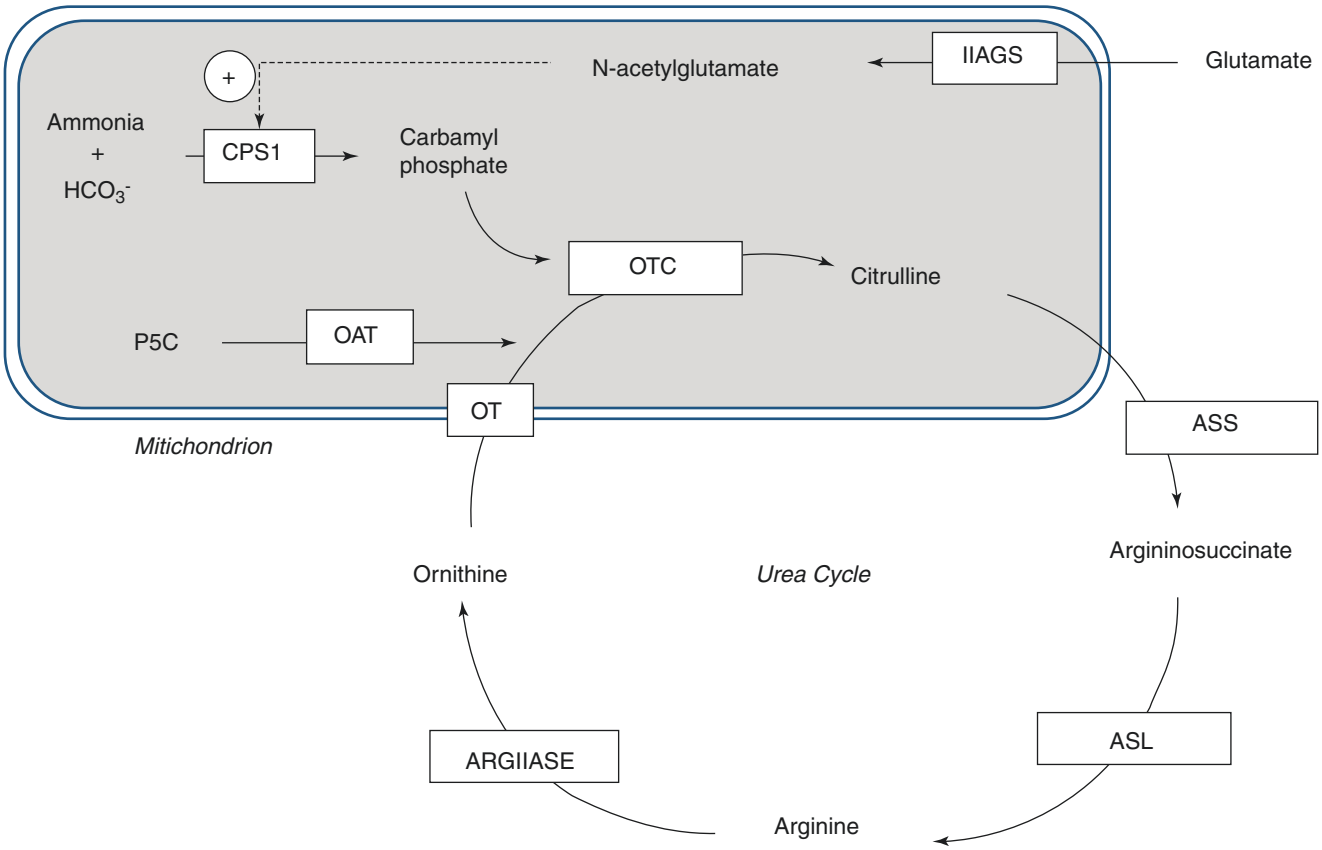

Fig. 67.4 Urea cycle disorders. *CPS* carbamoyl-phosphate synthetase 1, *OAT* ornithine aminotransferase, *OT* ornithine transferase (hyperornithinaemia, hyperammonaemia, homocitrullinuria or HHH syndrome), *OTC* ornithine transcarbamylase, *NAGS N*-acetylglutamate synthase, *ASS* argininosuccinate synthase, *ASL* argininosuccinate lyase, *P5C* pyrroline-5-carboxylate

X-linked recessive. Females can present with symptoms due to the effects of lyonisation if sufficient wild-type OTC genes are inactivated in the liver. OTC is the most common UCD.

Neonatal presentation is a classical intoxication with lethargy/irritability, poor feeding and vomiting progressing to overt encephalopathy. Tachypnoea secondary to the respiratory stimulant effect of ammonia initially produces a mild respiratory alkalosis. Apnoea and fits are also common. First presentation may occur later with a variety of non-specific features including failure to thrive, developmental delay, anorexia and vomiting. Investigation of cyclical vomiting should include assessment of ammonia during at least one of the episodes. Children may appear fussy eaters self-electing a low-protein diet. Episodes of acute metabolic decompensation may be precipitated by intercurrent infections. Long-term neurological sequelae relate to the severity of encephalopathic episodes (peak ammonia and duration), with peak ammonias as low as 360 μmol/l being associated with poorer outcome [51].

Diagnosis is suggested by marked hyperammonaemia and low plasma urea in severe defects. Assessment of amino acids and urinary orotic acid reveals the level of the block (Table 67.5) and can be analysed on a single sample by tandem mass spectrometry. Glutamine tends to be elevated as it forms part of the hepatic nitrogen pool that feeds into the urea cycle, and arginine low as it becomes an essential amino acid in UCDs due to reduced flux through the cycle, with the exception of arginase deficiency. Liver dysfunction and coagulopathy are often featured in the acute stage. The main differential diagnosis for hyperammonaemia includes spurious (poor sampling technique), organic acidaemia, acute liver dysfunction and FAOs. Diagnosis is confirmed by liver enzymology, and more recently mutation analysis as the first-line, prenatal diagnosis can then be made available.

Acute management requires cessation of feeds, promotion of anabolism with 10% dextrose-based fluids with the addition of an insulin infusion if hyperglycaemia persists and removal of ammonia. Initial management of hyperammonaemia includes loading with alternate pathway drugs: sodium benzoate (250 mg/kg) conjugates with glycine from the hepatic nitrogen pool allowing excretion in the urine bypassing the urea cycle and sodium phenylbutyrate (250 mg/kg) conjugates with glutamine. This helps reduce the nitrogen load on the cycle and can be followed by a continuous infusion. Arginine becomes an essential amino acid with the exception of arginase deficiency and should be supplemented

Table 67.5 Diagnosis of urea cycle defects

Condition	Enzyme	Key amino acids	Orotic acid	Tissue enzymology
NAGS deficiency	*N*-acetyl-glutamate synthase	Glutamine ↑ Arginine-normal	Normal	Liver
CPS deficiency	Carbamoyl-phosphate synthase	Glutamine ↑ Arginine ↓	Normal	Liver
OTC deficiency	Ornithine transcarbamylase	Glutamine ↑ Arginine ↓	↑↑↑	Liver
Citrullinaemia	Argininosuccinate synthase	Glutamine ↑ Citrulline ↑↑↑	↑	Liver
Argininosuccinic aciduria	Argininosuccinate lyase	Glutamine ↑ Argininosuccinate ↑	↑	Red blood cell Liver
Arginase deficiency	Arginase	Glutamine ↑ Arginine ↑↑↑	↑↑	Red blood cell Liver

(150 mg/kg load) followed by a continuous infusion. Higher doses are required in citrullinaemia and argininosuccinic aciduria (300–500 mg/kg/day). Carglumic acid, an *N*-acetylglutamate synthetic analogue, is the specific treatment for *N*-acetylglutamate synthase (NAGS) deficiency [52]. A trial of carglumic acid has been proposed for use in all undiagnosed hyperammonaemic crises. If ammonia levels are >300 μmol/l and rising, dialysis is indicated for rapid control and to avoid the long-term neurological sequelae of prolonged hyperammonaemia. The latter can be achieved with hemofiltration but may be limited by the size of the baby (>2.5 kg) where peritoneal dialysis (PD) is used. Crossflow PD using two catheters may be used in the smallest babies to improve ammonia clearance [53].

Long-term management requires dietary protein restriction, titrating growth with ammonia (<50 μmol/l) and glutamine (<1000 μmol/l) concentration. Essential amino acids are monitored to ensure the adequacy of the diet. Essential amino acid supplements can be used as part of the protein intake having the advantage that nitrogen waste will be reduced as nonessential amino acids will need to be synthesised. Sodium benzoate and/or sodium phenylbutyrate and arginine continue as part of long-term management; Carglumic acid is used in NAGS deficiency and sometimes CPS1 deficiency. Citrulline can be added or substituted with arginine in severe OTC and CPS deficiency [54].

Prognosis is related to the peak ammonia and duration; a rapid reversal of hyperammonaemia reduces the risk of neurological injury. Liver fibrosis may develop in argininosuccinic aciduria, and the exact pathophysiology is not known; however, regular liver imaging and AFP monitoring is recommended. Neonatal presenting OTC has the poorest prognosis, and early liver transplantation in the first 6 months is indicated to offer the best long-term outcomes [55]. Liver transplantation in UCDs is well published with successful outcomes and improves quality of life [56]. Hepatocyte transplantation has been used as a bridge to transplant in some cases [57], and more recent trials for gene therapy are underway with potential good outcomes [58].

Citrin Deficiency

Citrin deficiency results from a deficiency of the glutamate–aspartate transporter which has a role in gluconeogenesis from lactate and transporting cytosolic nicotinamide adenine dinucleotide (NADH)-reducing equivalents into mitochondria as part of the malate aspartate shuttle. Citrin deficiency causes three age-specific phenotypes: neonatal intrahepatic cholestasis associated with citrin deficiency (NICCD), in older children as failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD) and in adults as recurrent hyperammonaemia with neuropsychiatric symptoms in citrullinaemia type II (CTLN2) [59]. The clinical features of NICCD are a transient intrahepatic cholestasis and poor weight gain. Liver dysfunction is variable and usually resolves in the first year of life. Transaminases are modestly elevated with hypoproteinaemia and reduced coagulation factors. Complications include haemolytic anaemia and hypoglycaemia. Ammonia is only mildly elevated, but citrulline and methionine are significantly elevated with milder elevations in threonine, tyrosine, lysine and arginine [60]. Diagnosis is confirmed by SLC25A13 genotyping. Management includes the supplementation of fat-soluble vitamins and the use of lactose-free or formulas containing medium-chain triglyceride (MCT) during the period of liver dysfunction. Many children subsequently develop aversion to carbohydrate-rich food and prefer protein-rich or fat-rich foods developing hyperlipidaemia and the risk of fatty liver and pancreatitis.

CTLN2 is characterised by acute-onset recurrent hyperammonaemia with neuropsychiatric symptoms. Hepatic argininosuccinate synthetase protein is reduced but without any mutations in the argininosuccinate (ASS) gene as seen in citrullinaemia. Precipitants include alcohol, sugar intake, medicines such as anti-inflammatories or analgesics or surgery. Not all patients have a preceding history of NICCD. Liver transplantation is the most effective treatment, preventing hyperammonaemic crises and reversing the

biochemistry [61]. Careful dietary supervision is required to provide appropriate carbohydrate (low) and protein (high) intake as the standard hyperammonaemic treatment. Intercurrent illness and low protein with high carbohydrate can precipitate crises in these patients. This is because carbohydrate suppresses ureagenesis in citrin deficiency. Emergency regimens consist of high protein and low carbohydrate. Sodium pyruvate may have a role in treatment by reducing oxidative stress, reducing NADH and reducing inhibition of ureagenesis [62].

Lysinuric Protein Intolerance (LPI)

LPI is a rare disorder resulting from recessive inherited mutations involving the *SLC7A7* gene. Defects occur in the y^+LAT1 subunit of the cationic amino acid transporter localised at the basolateral membrane of the tubular kidney and small bowel cells, leading to the classical hallmarks of the disease: leakage of cationic amino acids in the urine (arginine, ornithine, lysine) with associated normal to low plasma levels. y^+LAT1 is also expressed in the lung and spleen and in circulating monocytes and macrophages, which would explain the wide spectrum of symptoms that has been described, such as failure to thrive, protein intolerance, hepatosplenomegaly, osteoporosis, lung involvement, kidney failure, immunological disorders with autoimmunity and haemophagocytic lymphohistiocytosis. Plasma lysine, ornithine and arginine are low with corresponding increases in urinary excretion. Plasma glutamine, alanine and glycine can be elevated. Ammonia can be elevated, particularly postprandially due to arginine depletion and secondary urea cycle dysfunction. Lactate dehydrogenase, ferritin and hyperlipidaemia are common. In the acute situation treatment with arginine and ammonia, scavenging agents may be required for hyperammonaemia. Long-term treatment requires protein restriction, citrulline and lysine supplementation and ammonia scavengers. Periodic evaluation of renal function, lung involvement, serum LDH and ferritin and plasma amino acids is recommended [63, 64].

Organic Acidaemias

Organic acidaemias result from inherited blocks in the catabolic pathways of amino acids provoking the accumulation of organic acids prior to the block. There are many disorders, but the most common are propionic acidaemia (PA), methylmalonic acidaemia (MMA) and isovaleric acidaemia (IVA). Acute presentations, either severe neonatal or intermittent late-onset forms, are associated with encephalopathy, marked metabolic acidosis, raised anion gap, ketosis, elevated lactate and variable hyperammonaemia, the latter resulting from secondary inhibition of the urea cycle. Hepatomegaly and liver dysfunction are frequent. Neutropenia, or frank pancytopenia, is a sign of marrow depression and may lag behind the initial presentation and will recover as the decompensation is controlled. Blood glucose may be low, high or normal. Hypocalcaemia is a frequent finding in the acute stage. IVA has an associated pungent odour of sweaty feet.

Diagnosis relies on urinary organic acid analysis to identify the key urinary metabolites and acylcarnitine analysis to identify abnormal plasma metabolites. Free carnitine may be depleted, and glycine is often raised on plasma amino acid analysis. Complications include pancreatitis, cardiomyopathy and renal failure. Amylase should be measured during metabolic crises. Optic neuropathy has more recently been recognised as an association in PA and MMA [65]. Liver histology may show macro- or microvesicular fatty infiltration. Liver tumours have been reported in a few cases of MMA [66]. Definitive diagnosis is with genetic confirmation, *PCCA* and *PCCB* in PA and *Mut*, *CblA* and *CblB* in MMA.

Acute management is focused on promoting anabolism while removing toxic metabolites—intravenous fluids containing 10% dextrose are used in conjunction with protein restriction. Carnitine supplementation is used to facilitate organic acid excretion. Glycine is used in IVA for conjugation and increased clearance. Alternate pathway medication is used to manage acute hyperammonaemia, and recently carglumic acid has been shown to be of use in some patients. Theoretically, the site of urea cycle inhibition is at the level of NAGS, and carglumic acid is a synthetic analogue of *N*-acetylglutamate [67]. Acute toxin removal during crises may require dialysis.

General management consists of protein restriction to a level that prevents overload of the pathway while providing sufficient for growth and repair, with or without amino acid supplementation, and cofactor supplementation. A trial of vitamin B_{12} should be given to assess responsiveness in all patients with MMA. Metronidazole in low dose either continuously or episodically may be used to alter bowel flora to reduce propionate production from the gut. Constipation in itself may precipitate decompensation due to increased gut propionate production [68].

Patients remain at risk of decompensation most commonly precipitated by intercurrent infection, and for patients with neonatal presentations, the outlook is poor [69]. Neurological and cognitive defects are common, and progressive renal impairment is a feature of MMA leading to renal failure in the second to third decade. MMA patients who are B_{12} responsive have a better prognosis, but the poor outcomes for most organic acidaemias have led to serious consideration of transplantation. Liver transplant in PA has been successful with a marked reduction in risk of decompensation, but the risk of metabolic stroke persists, and so a

degree of protein restriction may still be required [70]. The value of liver transplantation in MMA is less certain with significant continued risk of metabolic stroke and renal involvement [71]. Combined liver and renal transplantation has been performed with variable outcomes, and isolated renal transplant may also have a role in MMA patients with chronic kidney disease and may help reduce the frequency of decompensation [72].

Disorders of Lipid Metabolism

Fatty Acid Oxidation Defects (FAOD)

The FAODs for a large group of conditions commonly present with hepatic symptoms. Fatty acids are a major fuel source in the fasted state, the preferred substrate for cardiac muscle and a vital energy source for skeletal muscle during prolonged exercise. Fatty acids are mobilised from stores and are oxidised by most tissues except the brain, which is reliant on hepatic β-oxidation for ketone production that can be utilised within the central nervous system reducing the demand for glucose. Fatty acids are predominantly oxidised within mitochondria (Fig. 67.5 and Table 67.6). The carnitine cycle is required for passage of fatty acyl-coenzyme A (CoA) across the mitochondrial membrane with the exception of medium-chain fatty acids. The fatty acyl-CoAs then enter the β-oxidation cycle with shortening of the fatty acid by two carbons with each passage through the cycle yielding acetyl-CoA for entry into the TCA cycle or ketone production (liver) and reduced cofactors for entry into the respiratory chain for ATP production. The acyl-CoA dehydrogenases central to the β-oxidation cycle are chain length specific, for example, short-chain acyl-CoA dehydrogenase (SCAD), medium-chain acyl-CoA dehydrogenase (MCAD) and very-long-chain acyl-CoA dehydrogenase (VLCAD) for saturated straight chain fatty acids. Unsaturated fatty acids require additional enzymes for oxidation, while branched-chain fatty acids require initial α-oxidation in peroxisomes before they can enter β-oxidation. This is why very-long-chain fatty acids (VLCFA, which include the branched-chain fatty acids) are used as a screening test for peroxisomal disorders. Deficiencies of the electron transfer flavoprotein and its dehydrogenase (multiple acyl-CoA dehydrogenase deficiency, MADD, or glutaric aciduria type 2) block the transfer of electrons from β-oxidation to the respiratory chain and

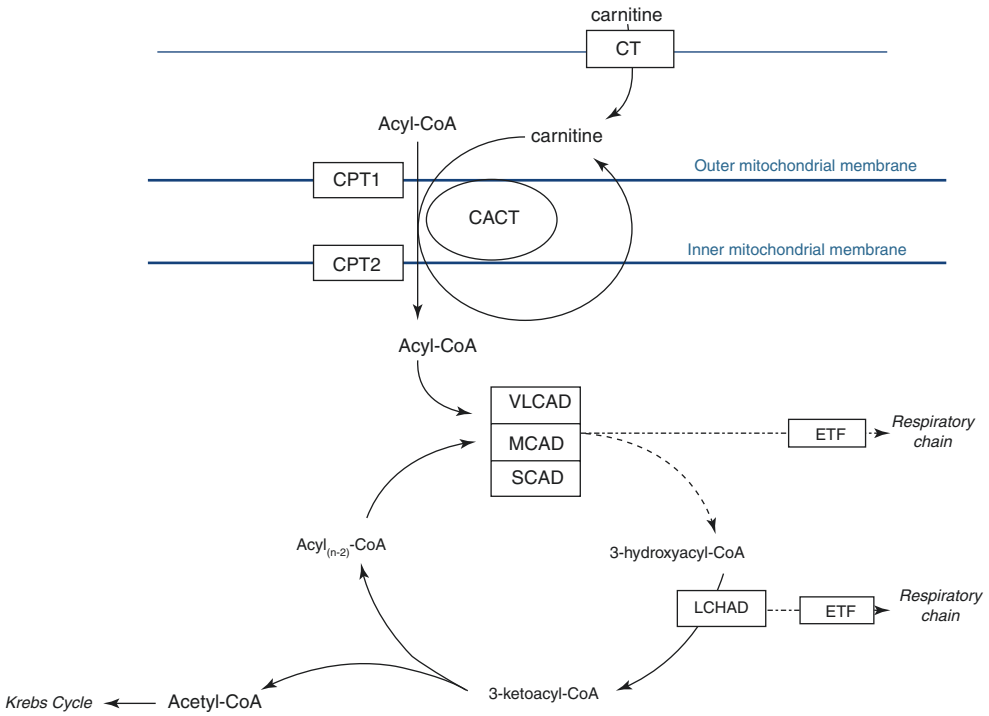

Fig. 67.5 Schematic of mitochondrial fatty acid β-oxidation. The carnitine shuttle facilitates fatty acid entry into the mitochondrial matrix where the β-oxidation spiral chain shortens fatty acids releasing energy in the form of acetyl-CoA that enters the Krebs cycle and electrons that are transferred via electron transfer flavoproteins *(dash-dot line)* to the respiratory chain. Disease-causing enzymes are boxed: *CT* carnitine transporter, *CPT1* carnitine palmitoyltransferase 1, *CPT 2* carnitine palmitoyltransferase 2, *CACT* carnitine acylcarnitine translocase, *VLCAD* very-long-chain acyl-CoA dehydrogenase, *MCAD* medium-chain acyl-CoA dehydrogenase, *SCAD* short-chain acyl-CoA dehydrogenase, *ETF* electron transfer flavoproteins (causing multiple acyl-CoA dehydrogenase deficiency (MADD)), *LCHAD* long-chain hydroxyacyl-CoA dehydrogenase

Table 67.6 Clinical features of fatty acid oxidation defects

Enzyme/transporter defect	Hypoketotic hypoglycaemia/liver dysfunction	Cardiomyopathy	Rhabdomyolysis	Other features
Carnitine cycle				
Carnitine transporter (CTD)	+	+		
Carnitine palmitoyltransferase-1 (CPT1)	+	+	+	Renal tubular acidosis
Carnitine/acylcarnitine translocase (CACT)	+	+		
Carnitine Palmitoyltransferase-2 (CPT2)	+	+ (CPT2 and cardiomyopathy)	+ (CPT2 and rhabdomyolysis)	
β-Oxidation cycle				
Very-long-chain acyl-CoA dehydrogenase (VLCAD)	+	+	+	
Long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) and trifunctional protein (MTP)	+	+	+	Retinopathy, neuropathy
Medium-chain acyl-CoA dehydrogenase (MCAD)	+	+	+	
Short-chain hydroxyacyl-CoA dehydrogenase (SCHAD)	+			
Electron transfer				
Multiple acyl-CoA dehydrogenase (glutaric acidurias type II)	+	+		

also block the oxidation of branched-chain amino acids, lysine and glutaric acid.

Clinical features are predominantly hypoketotic hypoglycaemia, encephalopathy, hepatomegaly, cardiomyopathy and rhabdomyolysis with the majority presenting within the first year, often precipitated by intercurrent infection. Fasting tolerance increases with age as hepatic glycogen stores increase and relative glucose requirement decreases. Hepatic symptoms are common with associated elevated lactate and ammonia. Acute hepatomegaly can occur due to fatty acid mobilisation. Liver failure can occur. Hepatic symptoms may also occur in carrier mothers carrying an affected foetus. Cardiac involvement, either cardiomyopathy or arrhythmias, may be seen in all FAOs except carnitine palmitoyltransferase 1 (CPT 1). Episodic muscle pain, rhabdomyolysis and myoglobinuria may be precipitated by metabolic stress such as exercise or intercurrent infections. Creatine kinase is elevated during muscle crises. Proximal muscle weakness may have a more chronic progressive course. Neuropathy and pigmentary retinopathy are long-term complications of LCHAD deficiency. Neurological deficits may remain following acute encephalopathic insults in many of the FAODs.

Inappropriately low ketones in the presence of hypoglycaemia always raise the possibility of an underlying FAO. Plasma acylcarnitine analysis by tandem mass spectrometry is the investigation of choice to diagnose the specific defect. Plasma free carnitine may be depleted, thereby masking the diagnostic acylcarnitine profile. Very low free carnitine levels are found in a carnitine transport defect and are significantly elevated in CPT I deficiency. Full characterisation may require fat oxidation studies on cultured fibroblasts. Urinary organic acids at the time of decompensation may reveal characteristic dicarboxylic acids; however, they may be normal in between episodes. Further confirmation may be gained from genotype analysis screening for the common A985G mutation in MCADD and G1528C in LCHAD. FAODs are inherited in an autosomal recessive fashion, and therefore siblings should be screened.

The acute management consists of glucose either orally or, if not tolerated, intravenously as 10% dextrose saline in order to halt lipolysis. Hypoglycaemia is a late sign, and so normoglycaemia does not exclude significant decompensation, and therefore management should not be delayed. Resolution of encephalopathy may take some hours. Long-term management consists of avoiding prolonged fasting. An emergency regime consisting of giving frequent high-calorie glucose polymer drinks every 2 hours day and night is adopted during intercurrent infections. Failure to improve or to tolerate the drinks requires admission to hospital for intravenous dextrose.

In the long-chain defects, fat mobilisation is suppressed to avoid the production of toxic long-chain acylcarnitines by frequent feeds, the use of uncooked cornstarch and overnight nasogastric feeds. Long-chain fat in the diet is restricted, and essential fatty acids supplemented as walnut oil. MCT bypasses the carnitine cycle and enters the mid-portion of the β-oxidation cycle and therefore may be of benefit in carnitine cycle and long-chain defects.

Carnitine replacement is essential in the carnitine transport defects (100–200 mg/kg/day). Low levels of carnitine are seen in many FAODs due to accumulation of acylcarni-

tines. The use of carnitine in long-chain FAOs remains controversial with the theoretical risk of compounding mitochondrial toxicity with the accumulation of long-chain acylcarnitines. A trial of riboflavin should be started in all patients with MADD. Triheptanoin is currently under investigation for use in FAOs to help replenish tricarboxylic acid intermediates and therefore increase flux and ATP production [73, 74].

The prognosis in many FAODs depends on the avoidance of acute decompensations. The outcome in MCADD is much improved once the diagnosis is made and future crises avoided. Neonatal screening for detection of MCADD in the UK and other FAOs overseas on blood spot acylcarnitines by tandem mass spectrometry has greatly reduced morbidity and mortality but cannot eliminate early neonatal deaths prior to the screening result being available [75]. Siblings with milder phenotypes with the same genotype have been recognised since screening, particularly with regard to VLCAD.

Acyl-CoA Dehydrogenase 9 (ACAD 9)

Mitochondrial acyl-CoA dehydrogenase family member 9 (ACAD9) is essential for the assembly of mitochondrial respiratory chain complex I. ACAD9 is most homologous to VLCAD. Both ACAD9 and VLCAD function as homodimers associated with the inner mitochondrial membrane and catalyse the initial step of the fatty acid oxidation cycle. The clinical phenotype is dominated by cardiomyopathy but continues to expand and includes liver dysfunction or liver failure, hypoglycaemia, muscle hypotonia, lactic acidosis and neurological symptoms. Most patients present in the first year of life. Muscle biopsy tends to show complex 1 deficiency, and diagnosis is confirmed by genetic analysis. Patients may be responsive to riboflavin supplementation [76].

Glycerol-3-Phosphate Dehydrogenase 1 Deficiency (GPD1)

GPD1 encodes the glycerol-3-phosphate dehydrogenase, a cytosolic protein which catalyses the reversible redox reaction of dihydroxyacetone phosphate (DHAP) and reduces NADH to glycerol-3-phosphate and NAD^+; this plays a critical role in both carbohydrate and lipid metabolism. This rare condition causes transient infantile hypertriglyceridemia, hepatomegaly, hepatic steatosis and fibrosis. Differential diagnoses often include GSDs and LALD. There are very few cases worldwide, and associated hypoglycaemia and hypertriglyceridemia persisting into adulthood have been reported. The phenotype is expanding, and the long-term outcome is unclear. There are no other specific biochemical markers, and the diagnosis is most often made by genetic testing following clinical suspicion or broader testing with WES. GPD1 should be considered in the differential diagnosis of hepatic steatosis [77]. Treatment with fenofibrate has been reported in a single patient [78].

Lysosomal Storage Disorders

The lysosome is an intracellular organelle that is a key component in cellular recycling and homeostasis. The LSDs are a large group comprising over 50 disorders with an estimated combined prevalence of approximately 1 in 8000 [79]. The fundamental pathological mechanism of these disorders is a single enzyme deficiency leading to defective degradation or transport of a complex molecule such as a mucopolysaccharide or sphingolipid. The consequence is a buildup of 'storage' material that impacts on normal cellular function and homeostasis. These conditions are commonly progressive and multisystemic, affecting systems to a highly variable degree. Musculoskeletal involvement can include hypotonia, dysostosis multiplex, cardiomyopathy and the classic course features of the mucopolysaccharidosis. Endothelial reticular involvement can include hepatosplenomegaly and bone marrow dysfunction. Neurological involvement is common and is the most devastating feature of LSDs, causing severe neurological regression. Hepatomegaly, usually associated with splenomegaly, is a common feature of LSDs. A list of LSDs that are associated with hepatosplenomegaly is shown in Table 67.7. Of all the LSDs, the lipid storage diseases (Niemann–Pick disease and cholesterol ester storage disease) are most commonly associated with liver involvement and are considered in more detail below.

Niemann–Pick Type C Disease (NPC)

NPC is a progressive and life-limiting autosomal recessive disorder caused by mutations in either the *NPC1* (accounts for 95%) or *NPC2* gene. Mutations in these genes are associated with abnormal endosomal–lysosomal trafficking, resulting in the accumulation of multiple tissue-specific lipids within lysosomes. The clinical spectrum of NPC ranges from a neonatal rapidly progressive fatal disorder to an adult-onset chronic neurodegenerative disease. In regard to hepatic involvement, the neonate typically presents with cholestasis and persisting hepatosplenomegaly or acute liver failure, hepatosplenomegaly and ascites, which can be fatal early on. Visceral symptoms, if present, usually precede neurological symptoms and occur in about 85% of patients. The age of onset of the first (beyond 3 months of life) neurological symptoms may predict the severity of the

Table 67.7 LSDs commonly associated with hepatosplenomegaly

Disorder	Associated features
GM1/GM2 gangliosidosis	Neurological regression, coarse facies, cherry red spot
Mucopolysaccharidoses	Coarse facial features, +/− skeletal dysplasia, neurological regression, cardiomyopathy
Fucosidosis	Neurological regression, seizures, coarse facies
Mannosidosis	Neurological regression, corneal clouding, coarse facies
Sialidosis type II	Neurological regression, coarse facies, cherry red spot, cardiomyopathy
Galactosialidosis	Coarse facies, neurological regression, skeletal dysplasia, spasticity, corneal clouding, cherry red spot
Niemann–Pick A	Neurological regression, failure to thrive, cherry red spot
Niemann–Pick B	Growth restriction, interstitial lung disease, normal intelligence
Niemann–Pick C	Neonatal liver disease, neurological regression, seizures, cherry red spot
Farber	Contractures, skin nodules, neurodegeneration
Multiple sulphatase deficiency	Coarse facies, skeletal dysplasia, neurodegeneration, spasticity
I-cell disease	Neonatal coarse features, skeletal dysplasia, neurodegeneration, cardiomyopathy
Pompe	Hypotonia, cardiomyopathy, macroglossia
Wolman (infantile CESD)	Failure to thrive, abdominal distension, diarrhoea, calcification of adrenal glands

disease and determine life expectancy. The most characteristic neurological symptoms are cerebellar ataxia, dysarthria, dysphagia and developmental delay or progressive dementia. The majority of patients show a characteristic vertical supranuclear gaze palsy. Pulmonary involvement is well documented in NPC [80].

Diagnosis can be difficult and prolonged. More recently, genetic analysis is the first line, and the NPC genes are included in cholestatic gene panels in some centres. Other biomarkers of disease include elevated chitotriosidase (marker of macrophage activity; however, it can be normal in 5% of the European population) and elevated plasma oxysterols [81]. Filipin staining demonstrates accumulation of unesterified cholesterol in perinuclear vesicles (lysosomes) after staining with filipin in cultured fibroblasts. This requires an invasive procedure and is time-consuming; however, it can be used in the event of finding variants of uncertain clinical significance [82].

There is no effective treatment for NPC and largely consists of supportive therapies [83]. Miglustat has been shown to slow the neurological progression of disease in some patients [84]. There are a number of agents undergoing clinical trials such as cyclodextrin [85].

Niemann–Pick A and B Disease

Acid sphingomyelinase deficiency (ASMD) is also known as Niemann–Pick types A and B. The metabolic defect in ASMD is deficiency of the lysosomal enzyme acid sphingomyelinase due to mutations in the sphingomyelin phosphodiesterase 1 gene (*SMPD1*). ASM catalyses hydrolysis of sphingomyelin to ceramide and phosphocholine, and deficiency results in the progressive accumulation of sphingomyelin and other lipids within tissues that are rich in reticuloendothelial cells, including the spleen, liver, lung, bone marrow and lymph nodes. The disease spectrum ranges from a rapidly progressive neurovisceral neonatal form (NPA), a chronic neurovisceral form (intermediate) and a chronic visceral form without neurological involvement, presenting with hepatosplenomegaly. Pulmonary function may worsen over time, and interstitial lung disease and pulmonary infections are common. A mixed dyslipidemia is seen early in the disease course, and some patients develop coronary artery disease later on in life. Development of hepatic fibrosis ranging from minimal to cirrhosis is common, and progression of liver disease contributes to early mortality in some patients. Progressive splenomegaly may result from deposition of sphingomyelin and progressive portal hypertension [86].

Cholesterol Ester Storage Disease (CESD)

CESD or lysosomal acid lipase deficiency (LALD) is a recessively inherited condition caused by mutations in the gene encoding lysosomal acid lipase, *LIPA*. The resulting reduced or absent enzyme activity leads to a spectrum of disease, from the severe infantile form (also known as the Wolman disease) to a later-onset milder form which can remain asymptomatic and undiagnosed into adulthood. The infantile form is a severe disorder that presents during infancy, resulting in failure to thrive, hepatomegaly, often splenomegaly and hepatic failure, adrenal calcification and an average life expectancy of less than 4 months. Cholesterol ester storage disorder arising later in life is less severe, with a heterozygous phenotype with hepatomegaly and transaminitis, chronic liver disease and cardiovascular disease. The two phenotypes share many common features, including dyslipidaemia and transaminitis. The dyslipidaemia shows an elevated total cholesterol and low-density lipoproteins (LDL) and low high-density lipoproteins (HDL). The diagnosis is confirmed by identification of either biallelic pathogenic variants in *LIPA* or deficient LAL enzyme activity in peripheral blood leucocytes, fibroblasts or dried blood spots [87].

In 2015, regulatory agencies approved the use of a human recombinant LAL for the treatment of LALD. This

long-term enzyme replacement therapy has been associated with significant improvements in the hepatic and lipid profiles of patients with LALD, increasing survival rates markedly in infants with an otherwise rapidly progressive disease [88, 89].

Disorders of Bile Acid Synthesis (BASD)

The synthesis of bile from cholesterol is a complex pathway consisting of at least 14 enzymatic reactions. Defects at specific enzymatic steps cause disease resulting in reduced bile flow and accumulation of abnormal and toxic bile acid intermediates. These disorders broadly tend to present with cholestasis, fat-soluble vitamin malabsorption and, in some subtypes, neurological involvement. They tend to present in infancy but can be diagnosed at any age. The diagnosis is made from the detection of urinary or blood metabolites characterising BASDs via mass spectrometry.

The most common BASD is 3β-hydroxysterol-C_{27}-steroid oxidoreductase (3β-HSD) deficiency, which presents in the neonatal period with a low GGT cholestasis, transaminitis, hepatomegaly and fat-soluble vitamin malabsorption. Liver biopsy shows cholestasis and giant cell transformation. The phenotype is heterogeneous, and some can show resolution of jaundice and present later on with liver dysfunction; the majority however go on to develop progressive cirrhosis without treatment. Treatment with cholic and chenodeoxycholic acid supplementation is successful [90].

Δ4–3-ketosteroid 5β-reductase deficiency is similar in presentation but tends to manifest at a younger age and is characterised by a more severe course with an elevated GGT and often coagulopathy.

Cerebrotendinous xanthomatosis (CTX) or 27-hydroxylase deficiency is a defect at the entry step of the classical bile acid synthetic pathway. It can present with neonatal cholestasis, progression to liver failure or in adulthood with CNS and tendon xanthomas and ataxia. Long-term treatment with chenodeoxycholic acid (750 mg/d) can improve neurological symptoms and contribute to a better prognosis [91].

Bile acid amidation defects are two defects responsible for the lack of bile acid conjugation with amino acids glycine and taurine, respectively. Both can present with low GGT cholestasis and fat-soluble vitamin malabsorption. Treatment with glycocholic acid has been shown to be beneficial.

2-Methylacyl-CoA racemase deficiency has been reported in very few patients who can present with cholestatic liver disease in infancy or fat-soluble vitamin malabsorption in older patients [92]. Treatment of the neuropathy is with a phytanate restricted diet, while the neonatal cholestasis may respond to cholic acid therapy. It should be noted VLCFAs will be normal with elevated pristanic and bile acid intermediates dihydroxy cholestenoic acid (DHCA)/trihydroxy cholestenoic acid (THCA).

Peroxisomal Disorders

Peroxisomes are subcellular organelles present in all cells, except mature erythrocytes, and are especially abundant within the liver. Key functions include β-oxidation of chain fatty acids and fatty acid derivatives, pipecolic acid and glyoxylate degradation, phytanate α-oxidation, plasmalogen, cholesterol and bile acid biosynthesis [93]. Biochemically, peroxisomal disorders are characterised by the extent of peroxisomal dysfunction from complete absence of peroxisomes (biogenesis disorders) to multiple enzyme defects and single enzyme defects. However, there is much overlap within the phenotypes of these groups; hence, a more useful categorisation is made by considering clinical features and age of presentation.

In the most severe peroxisomal phenotype, Zellweger spectrum disorder (ZSD—includes previous classifications of Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease), hepatic involvement is a prominent feature. ZSD presents in the neonatal period or early infancy with severe neurological impairment including marked hypotonia, seizures and neurological impairment, jaundice, hepatomegaly, cholestasis, liver dysfunction and classical dysmorphic features (prominent forehead, large fontanelles). Liver histology can show fibrosis, which may develop into micronodular cirrhosis. Renal cysts are common; however, they may not be easily demonstrable in ultrasonography. Ocular features are common including retinopathy, cataracts and optic nerve dysplasia. Failure to thrive evolves and little developmental progress is made. Death usually occurs within the first 12 months although the more attenuated forms may involve survival well into the first decade.

Defects in a large number of genes have been described as causing ZSD (mostly *PEX* genes). Initial investigations should include plasma VLCFAs. These are typically elevated, but it should be noted that in some peroxisomal phenotypes other than ZSD VLCFAs may be normal. Further delineation of the biochemical block may be gained by measuring plasma bile acid intermediates and erythrocyte plasmalogens. Definition of the precise molecular defect requires skin biopsy and complementation studies followed by enzymology or molecular genetic techniques. Other investigations that may be considered to support a diagnosis of ZSD are electroretinogram (ERG), visual evoked potentials (VEPs) and brain auditory evoked responses (BSAERs) that are abnormal in most cases. Neuroimaging may delineate neuronal migrational defects and demyelination. Plain films of the knees may reveal calcific stippling. More recently, an

increasing number of patients are being diagnosed by WES or WGS as first-line testing followed by more detailed fibroblast studies for confirmation of potentially causative variants.

A newly described peroxisomal disorder involving the liver is peroxisomal *ABCD3* (*PMP70*) deficiency. ABCD3 also known as PMP70 uses one of the three ATP-binding cassette transporters present in the peroxisomal membrane and catalyses the ATP-dependent transport of substrates for metabolic pathways into the peroxisome. The first patient presented with hepatosplenomegaly and severe liver disease with markedly elevated di- and trihydroxy cholestenoic acid [94].

Treatment remains supportive and can be challenging. Seizures are typically difficult to control, and hypotonia often results in a requirement for supportive feeding. Progressive liver impairment often contributes to morbidity and mortality. Vitamin K supplementation to correct coagulopathy and treatment of any associated adrenal insufficiency are recommended. Docosahexaenoic acid (DHA) supplementation has been attempted as it is believed to play an important role in brain development, but most recent evidence does not support therapeutic benefit [95, 96]. Ursodeoxycholic acid is commonly used to improve bile flow in cholestatic patients, but the primary bile acids cholic and chenodeoxycholic acids have also been reported to improve hepatic outcome in those without advanced liver disease. The primary bile acids are postulated to improve bile flow but also inhibit, by negative feedback, the production of abnormal bile acid metabolites formed in the absence of functioning peroxisomes.

Mitochondrial Disorders

Mitochondria are ubiquitous intracellular organelles with complex roles in intermediary metabolism and cell signalling. Functions include ATP generation via oxidative phosphorylation (OXPHOS) system, which consists of five enzyme complexes (I–V), homeostasis of intracellular calcium levels, reactive oxygen species-mediated cell signalling and regulation of apoptosis.

Mitochondrial disease can affect any organ and present at any age with any symptom. Organs with high energy demand tend to produce the majority of symptoms: brain, liver, muscle, heart and kidney. Gastrointestinal and hepatic manifestations are frequent; however, they are rarely isolated symptoms and occur as part of a multisystem disease. Diagnostic clues such as lactic acidaemia may be present but are often absent, and further evidence of mitochondrial dysfunction is often sought by biochemical assay of respiratory chain enzymes that require invasive muscle biopsy. Cerebrospinal fluid lactate can be useful for detecting CNS involvement. Multisystem involvement should be sought if clinical suspicion is raised. Genetic inheritance of mitochondrial disorders is complex and relies on the synergistic relationship of two unique genomes: nuclear DNA (nDNA) defects (usually autosomal recessive) or mitochondrial DNA (mtDNA, matrilineal inheritance) in the form of point mutations or large deletions and rearrangements. Nuclear DNA defects affect the structure and function of mitochondria. The mtDNA depletion syndrome is caused by defects in nDNA, which controls mtDNA replication, stability and maintenance. Hepatocytes contain thousands of molecules of mtDNA, and mostly their sequence is identical, i.e. homoplasmy. However, mutated mtDNA can co-exist with their wild-type counterpart in various proportions, i.e. heteroplasmy, and this has implications for widely diverse phenotypes of mitochondrial disease.

Treatment for the vast majority of mitochondrial disorders remains symptomatic and supportive. Most are progressive disorders, while examples of self-limiting conditions have recently been described. There are a rapidly increasing number of mitochondrial disorders being described whose pathophysiology is becoming clear and that present with well-defined clinical syndromes. Examples of these that include the hepatic/GI system are included in Table 67.8, and the reader is directed to several recent reviews of hepatic/GI mitochondrial disease [97, 98]. Any review of mitochondrial disease warrants a text all to itself, but this section outlines a few of the mitochondrial conditions whose primary features are hepatic/GI in nature.

Hepatic dysfunction is common in mitochondrial DNA depletion syndromes (MDDS). These conditions may present with early-onset liver failure, hepatomegaly and hypoglycaemia and are usually associated with significant neurological symptoms such as seizures, encephalopathy, neuroregression and nystagmus. Alpers (Alpers–Huttenlocher) syndrome typifies this presentation and is caused by mutations in the nuclear gene *POLG* that encodes a DNA polymerase that is responsible for mitochondrial replication. Seizures are often the herald of disease onset, usually before the age of 2 years. Liver failure may then ensue and can be triggered by valproate therapy. Hepatic dysfunction has also been associated with disorders of mitochondrial DNA translation. A particularly interesting example of this is seen in mutations of the *TRMU* gene. These are associated with a syndrome of acute liver failure in early infancy that, if surviving the initial presentation, has spontaneous recovery of liver function and subsequent normal development with favourable prognosis. Identification and intensive support of these patients is paramount. Defects of individual respiratory chain complexes can also give rise to hepatic disease. An example of this is GRACILE syndrome (growth restriction associated with aminoaciduria, cholestasis, iron overload, lactic acidosis

Table 67.8 Mitochondrial syndromes affecting hepatic/gastrointestinal systems

Disorder	Clinical features	Gene(s)
Alpers–Huttenlocher syndrome	Liver failure and seizures	*POLG (PEO1, FARS2)*
Hepatocerebral MDDS	Liver failure, rotatory nystagmus, developmental delay, elevated plasma tyrosine	*DGUOK*
Hepatocerebral MDDS	Liver failure, chronic liver disease, peripheral neuropathy	*MPV17*
Succinyl CoA ligase deficiency	Liver failure, Leigh-like encephalopathy	*SUCLG1*
'Benign reversible' mitochondrial hepatopathy	Liver failure with spontaneous recovery	*TRMU*
Complex III deficiency	Liver failure and renal tubulopathy	*BCS1L*
GRACILE syndrome	Growth restriction, amino aciduria, cholestasis, iron overload, lactic acidosis and early death	*BCS1L*
MEGDEL syndrome	Liver disease with sensorineural hearing loss, Leigh-like encephalopathy and 3-methylglutaconic aciduria	*SERAC1*
Pearson marrow–pancreas syndrome	Exocrine pancreatic insufficiency and sideroblastic anaemia	Large scale mtDNA rearrangement
MNGIE	Pseudo-obstruction and peripheral neuropathy	*TYMP*
Ethylmalonic encephalopathy	Diarrhoea, acrocyanosis and petechiae	*ETHE1*

MDDS mitochondrial DNA depletion syndrome, *mtDNA* mitochondrial DNA, *MMA* methylmalonic acid, *GRACILE* growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death, *MEGDEL* methylglutaconic aciduria with deafness, encephalopathy and Leigh-like syndrome, *MNGIE* mitochondrial neurogastrointestinal encephalomyopathy

and early death) caused by mutations in *BCS1L*, although this has been linked to a variety of other phenotypes, many of which include liver impairment.

Examples of mitochondrial disorders that present with GI features include mitochondrial neurogastrointestinal encephalopathy (MNGIE) and ethylmalonic encephalopathy (EME). MNGIE is caused by an autosomal recessive deficiency of thymidine phosphorylase that classically presents in the first three decades of life. Initial symptoms are often related to GI dysmotility and can present as frank pseudo-obstruction and failure to thrive/cachexia. However, neurological symptoms develop that include peripheral neuropathy, ptosis, ophthalmoplegia, hearing loss and a progressive leukoencephalopathy. Lactic acidaemia may be present. Unusually for a mitochondrial disorder, a diagnostic metabolic profile of raised urinary/plasma thymidine and deoxyuridine will confirm the diagnosis. EME also has a characteristic metabolic profile with large amounts of ethylmalonic acid found in urine (ethylmalonic acid is commonly found in small amounts in urine as a non-specific marker of mitochondrial dysfunction). It is caused by mutations in *ETHE1* that encode a mitochondrial sulphur dioxygenase, which has a role in sulphur detoxification. Accumulation of hydrogen sulphide potently inhibits complex IV of the respiratory chain. Affected infants classically have persistent diarrhoea and the unusual finding of petechiae and acrocyanosis. Associated neurological findings include seizures, encephalopathy and developmental regression. This condition is usually lethal in infancy. Successful liver transplantation has been reported [99].

Investigation of suspected mitochondrial disease includes assessment of potentially affected organ systems; it is important to consider markers of renal tubular dysfunction, echocardiogram/ECG and neuroimaging. Non-specific biochemical evidence of mitochondrial dysfunction can be demonstrated by elevated plasma and CSF lactate levels, but further biochemical evaluation requires invasive testing such as muscle biopsy to assess individual components of the respiratory chain. The approach to genetic testing can be guided by muscle biopsy RCC deficiencies; however, more recently, WES or WGS may be able to identify a pathogenic variant in the nDNA or mtDNA in order to avoid invasive testing. Rapid testing for common nDNA genes causing MDDS is more readily available in some centres and can avoid unnecessary liver transplantation in cases where acute liver failure may require urgent decision-making [100].

Mitochondrial conditions have a vast array of presentations that can include hepatic dysfunction and GI symptoms. These are multisystem disorders and should always be in the differential diagnosis in a child with multisystem disease, especially a child with unexplained neurological symptoms and liver disease.

Conclusions

Inherited disorders of metabolism affecting the liver are individually rare but as a group of conditions are relatively common. The description of liver-based IMDs continues to expand particularly with the advent of novel molecular diagnostics and the availability of rapid metabolite detection. The liver can be predominantly involved or as part of a multisystem disease. The awareness of IMDs in the differential diagnosis of liver disease is vital. Developments in the understanding of the pathophysiology and genetics of these complex disorders will aid research into current and future novel therapies.

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Wilson's Disease

68

Piotr Socha and Stuart Tanner

Introduction

In 1912 Samuel Kinnier Wilson described adults with "Progressive lenticular degeneration, a familial nervous disease associated with cirrhosis of the liver." Recognition of the role of copper in Wilson's disease (WD) and the mainly hepatic presentation of WD in childhood came later [1]. After 100 years we know a lot about the disease – its treatment with copper chelators, diagnostic methods, its molecular basis, and the role of liver transplantation. Basic questions remain, such as an explanation for its phenotypic diversity. Moreover, diagnostic and therapeutic practices differ between countries as shown in a large European database of new Wilsonian cases within the "Eurowilson" project. A number of guidelines have appeared [2, 3], and recently a pediatric position statement of ESPGHAN has been published [4].

Definition

Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism, which leads to copper accumulation in the liver and other organs and tissues including the brain. The clinical presentation was first described by Wilson in 1912, who also indicated inheritance of the disease showing familial nature. The disease gene *ATP7B* encodes adenosine triphosphatase 7B (ATPase7B) – a protein responsible for transporting copper into the secretory pathway for incorporation into apoceruloplasmin and excretion into the bile. It is located on chromosome 13. Prevalence of different mutations differs among populations. Clinical presentation is variable – the disease commonly affects the liver and central nervous system. The prevalence of the disease is estimated to be 1:40000 up to 1:30000.

Pathophysiology

Copper is an essential dietary nutrient which is present in most of dietary products. The daily requirement is about 0.9 mg and the excess must be excreted. Copper facilitates electron transfer reactions and is needed for mitochondrial respiration, melanin biosynthesis, dopamine metabolism, iron homeostasis, antioxidant defense, connective tissue formation, and peptide amidation. Still, it can be potentially toxic to the cells which may be explained partly by the Fenton reaction in which Cu^{1+} causes production of the highly reactive hydroxyl radical which may cause lipid peroxidation of macromolecules:

$$H_2O_2 + Cu^{1+} > OH^{\cdot} + Cu^{2+} + H_2O.$$

Biliary excretion is the only mechanism for copper elimination under physiological conditions, and it increases with increasing size of the hepatic copper pool. Trafficking of copper in and through the hepatocytes involves several transport proteins: *Copper transporter 1* (Ctr1) responsible for the copper uptake at the hepatocyte plasma membrane; *metallothioneins* (MT), intracellular proteins capable of binding metal ions, including copper; and *metallochaperones* that mediate the delivery of copper to specific proteins and are responsible for transfer of copper from metallothionein to the site of synthesis of copper-containing proteins. Trafficking of copper is essential for further binding with ATP7B protein.

The Wilson's disease protein, ATP7B, has got several functions that finally allow effective copper excretion from

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the hepatocyte and binding with ceruloplasmin which protects from potential copper toxicity. Copper transport into the trans-Golgi is initiated by ATP binding to the "nucleotide-binding domain" of ATP7B. ATP hydrolysis and subsequent dephosphorylation allow the movement of copper associated with two binding sites on one of the transmembrane domains into the lumen of the trans-Golgi. Entering trans-Golgi is essential for further transport of copper – it is incorporated into apoceruloplasmin and other apoproteins and then excreted to sinusoids or after saturation of six copper-binding sites at the N-terminal cytoplasmic tail of ATP7B and phosphorylation of serine residues it migrates in vesicles to the biliary canaliculus [5].

Mutations of the WD gene coding ATP7B protein impair its function and lead to accumulation of copper in the hepatocyte and in other organs. The Human Gene Mutation Database [6] references 858 mutations, including 539 missense/nonsense, 71 splicing, 16 regulatory, and 146 small deletions. Large deletions are rare, in contrast to mutations in ATP7A (Menkes disease). H1069Q (His1069Glu) is the most common mutation in Europe and North America; the majority of WD patients are compound heterozygotes [7]. In central Europe, H1069Q occurs at allele frequencies of 30–60% in series of WD patients from Poland, Romania, Austria, and Saxony [8, 9]. By contrast, H1069Q is rare in Asian, Japanese, and Chinese patients, and in the heterogeneous population of the UK, WD is associated with many mutations, mostly compound heterozygous. Unusual genetic mechanisms such as three ATP7B mutations in a single patient (two in *cis* on the same chromosome) and uniparental disomy (both homologues of a chromosomal region originating from only one parent) emphasize the importance of also genotyping both parents. Several studies have identified families with WD in subsequent generations ("Pseudodominant" inheritance) emphasizing the need to consider the diagnosis of WD in the children of affected parents.

Genotypic incidence considerably exceeds phenotypic incidence. Whether this is due to underdiagnosis or incomplete penetrance is debatable [10].

The functional consequences of most mutations are not well described. H1069Q mutant binds ATP in an incorrect orientation with a reduced affinity, causing instability due to temperature-dependent unfolding and retention of $ATP7B_{H1069Q}$ within the endoplasmic reticulum [3]. For better understanding of the role of different mutation of functionality of ATP7B, a yeast strain that lacks its endogenous copper transporter was used to segregate ATP7B mutants into severe and mild categories based on their ability to restore growth. Studies in mammalian cells have shown decreased protein levels and mislocalization for several mutants, and copper transport has been studied in vesicles derived from cell lines with various ATP7B mutations [11]. The functional studies can help to understand why some mutations are pathogenic and lead to copper accumulation, but do not yet explain the phenotypic variability of Wilson's disease. Liver disease seems to result from a direct copper accumulation in hepatocytes, and consequently mitochondrial damage and disturbance of lipid oxidation. Hepatic steatosis is an early pathologic feature of the disease and may be explained by this mechanism. Injury of other tissues seems to be a consequence of copper accumulation outside the liver and usually appears at later age. Once capacity of the liver to store copper is exhausted, copper is released into the circulation. It can be taken by different organs, but the central nervous system is most vulnerable [12].

Specific pathways that allow the intracellular trafficking and compartmentalization of copper within the hepatocyte showing the role of ATPase7B are shown on Fig. 68.1.

Liver Histology and Ultrastructural Changes

Histological abnormalities observed in liver biopsy are not specific for Wilson's disease and cannot be regarded a valuable diagnostic tool. In the early phase, microvesicular and macrovesicular fatty deposition can be observed (Fig. 68.2), with glycogen-containing vacuoles in the nuclei of periportal hepatocytes. Liver disease may then progress to portal fibrosis and inflammation. It should be noticed that histological changes in WD may resemble those observed in autoimmune hepatitis with interportal fibrous bridging or cirrhosis.

Ultrastructural changes are also not regarded to have a significant diagnostic values – one of the typical features are pleomorphic mitochondria with increased matrix density and widening of intercristal spaces.

While rhodanine or rubeanic acid staining may demonstrate copper, the absence of histochemically demonstrable copper does not exclude a diagnosis of WD. Liver copper content >250 micrograms/g dry weight is a major diagnostic criterion – therefore, in suspected WD, a piece of the liver biopsy should be placed in a dry plastic copper-free container for subsequent analysis by atomic absorption analysis [2].

Clinical Symptoms

Wilson's disease may present with liver, neurological, or psychiatric symptoms. It seems that liver involvement can be observed in most of the neurological presentations, but it is

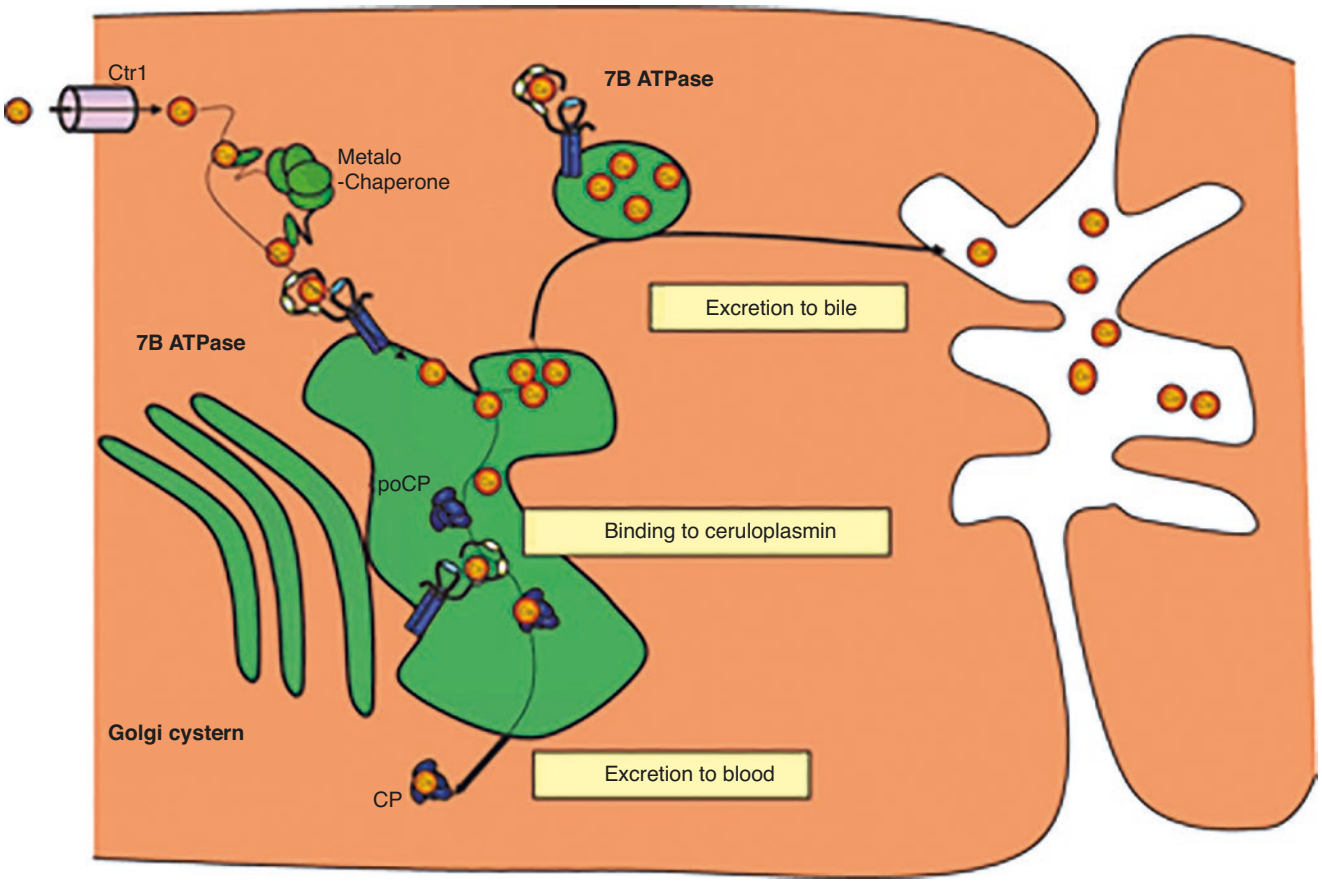

Fig. 68.1 Copper transport within a hepatocyte involving ATPase7B protein

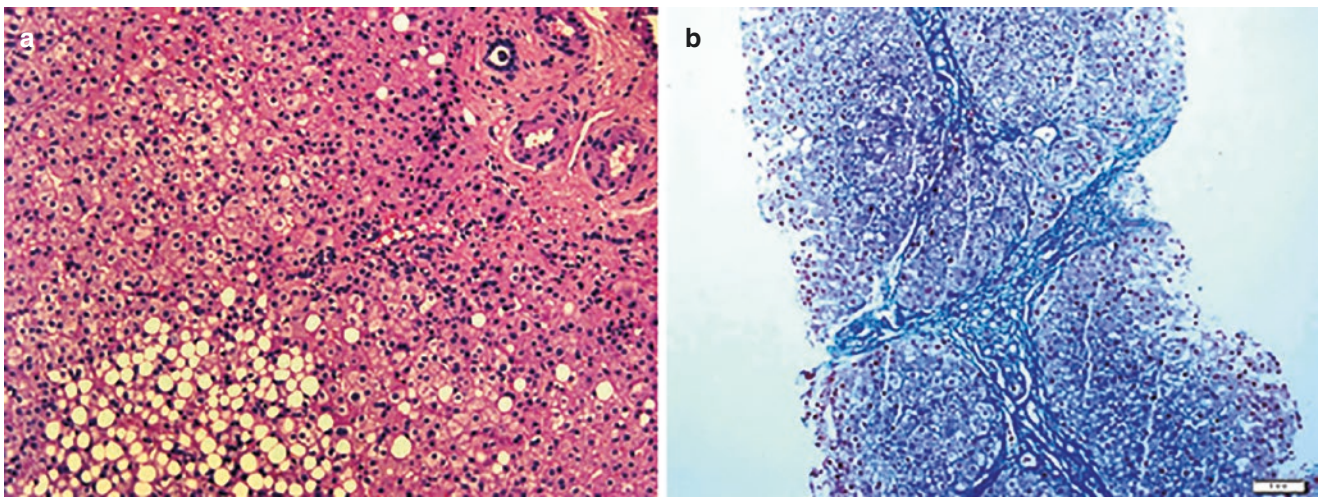

Fig. 68.2 Histopathological findings on liver biopsy from patients with Wilson's disease: (**a**) steatosis (HE staining) and (**b**) advanced fibrosis (Azan staining). (Courtesy of Dr Joanna Kuszyk, Department of Pathology of the Children's Memorial Health Institute, Warsaw)

not always looked for. Due to high index of suspicion in many countries, Wilson's disease is diagnosed very early in the presymptomatic phase. WD may present at any age from early childhood (>1 year of age, with raised transaminases, measured for some unrelated reason) to the eighth decade (often with surprisingly mild neurological features). Still, the clear liver symptoms (like hepatomegaly, clotting disturbances) have not been observed before the age of 2y [2].

Hepatic Symptoms

There is a wide spectrum of hepatic involvement from asymptomatic to hepatomegaly, fatty liver disease, hepatitis, jaundice, cirrhosis, and liver failure at the end. As indicated earlier the liver damage and liver symptoms are not characteristic, and making diagnosis required biochemical and/or molecular tests. Autoimmune hepatitis (AIH) can be easily misdiagnosed as specific for this condition autoantibodies are commonly found in Wilson's disease. Therefore, final diagnosis of AIH required exclusion of WD.

Acute liver failure is a severe presentation of Wilson's disease and requires fast diagnosis which may be difficult to obtain. It is usually found in a previously well child who suddenly presents with jaundice, hepatomegaly, rapidly progressing coagulopathy, and in some cases encephalopathy. The case history of a child may reveal important clinical problems in the past: episodes of jaundice, hemolytic anemia, or increased transaminases. Even if acute liver failure is not a chronic condition, liver biopsies taken at transplantation or post-mortem show cirrhosis which indicates that it is acute or chronic liver disease. Acute liver failure is defined according to the Paediatric Acute Liver Failure (PALF) study group – in a child with biochemical evidence of acute liver injury, international normalized ratio (INR) exceeds/equals 1 and in the presence of clinical hepatic encephalopathy or exceeds/equals 2.0 regardless of hepatic encephalopathy (HE). Wilson's disease makes a significant proportion of ALF etiologies – in 703 ALF patients in the PALF registry, 23 had a diagnosis of WD. However, among 329 patients without a final diagnosis, WD had been tested in only 81%, so there may be some underdiagnosis [13]. WD comprised 4% of the King's College Hospital pediatric ALF series [14].

Some clinical and lab features may indicate WD in acute liver failure like KF ring, family history of WD, neurological features of WD, jaundice and relatively moderately increased transaminases (100–500 IU/l) and alkaline phosphatase (<600 IU/l), hemolysis, and high bilirubin (>300 micromol/l) [15].

Presence of encephalopathy is a bad prognostic feature [16].

Chronic liver disease is more common among Wilsonian patients. Again, AIH may be falsely diagnosed because of the presence of low titer autoantibodies. The differential diagnosis requires also testing for viral hepatitis and alpha-1-antitrypsin deficiency.

Acute hepatitis is not commonly observed though some WD patients give a past history of jaundice and malaise from which they recovered.

Neurological Symptoms

Neurological symptoms are extremely rare in children with WD as they usually develop in the third decade of life and are the presenting symptoms of Wilson's disease in about half of all patients. Some initial symptoms can be difficult to be diagnosed like difficulty with speech. Otherwise, the neurological symptoms seem to be typical and can be described as:

1. A dystonic syndrome with dystonic postures and choreoathetosis.
2. An ataxic syndrome presenting as postural and intentional tremor and ataxia of the limbs.
3. A parkinsonian syndrome with hypokinesia, rigidity, and resting tremor.

To improve diagnostic approach and evaluate therapy, Czlonkowska and coworkers developed a scoring system for neurological symptoms in adult patients with Wilson's disease based on the Eurowilson project [17]. The limited data on neurological presentations in children does not allow to describe the frequency of neurological symptoms at this age. However, dysarthria, salivation, gait disturbances, and postural tremor should be looked for. The presence of Kayser-Fleischer ring is usually associated with neurological involvement [2].

Other Symptoms

Psychiatric symptoms are described to be the predominant presentation of Wilson's disease recognized in adults in later age but usually associate with other clinical symptoms. The most common seems to be depression – which can lead to attempted suicide (also from author's experience in children). Psychiatric problems are not easy to detect as some of them can be attributed to the teenage age like mood change, aggressiveness, and irritability. These symptoms may also be a reason for noncompliance with therapy once Wilson's disease is diagnosed.

Kayser-Fleischer ring is very typical for Wilson's disease and should be always looked for with a slit lamp – it is a gold or gray-brown opacity in the peripheral cornea which represents a deposit of copper and sulfur-rich granules in

Descemet's membrane. Although very characteristic, Kayser-Fleischer ring is uncommon in children.

Hemolysis with negative Coombs test is another typical feature of Wilson's disease even if not commonly described in children with Wilson's disease – usually it is associated with fulminant liver failure [2].

Dermatological findings are also described in children with Wilson's disease – like xerosis, keratosis pilaris, spider angioma, papulopustular lesions, and hyperpigmentation [18].

Fanconi syndrome (renal tubular abnormality presenting as glycosuria, aminoaciduria, renal tubular acidosis, impaired phosphate reabsorption) can be observed in the course of Wilson's disease. Proteinuria may also be detected pointing to glomerular damage in the course of Wilson's disease, but it is usually associated with penicillamine therapy.

Skeletal manifestations appear rarely and include arthritis, rickets, or osteoporosis.

Asymptomatic Wilson's Disease

Asymptomatic liver disease seems to be the most common presentation due to increasing awareness of Wilson's disease manifestation with slightly increased transaminases. Abnormalities at physical examination like hepatomegaly and splenomegaly and abnormal liver tests raise a suspicion of WD. In some countries liver tests are checked at many occasions, and any abnormalities lead to further differential diagnosis of liver problems [19]. Siblings of Wilsonian patients should be also diagnosed as early as possible usually in the preclinical phase.

Diagnostic Approach

Consider WD in any undiagnosed liver disease in childhood. As Wilson's disease can mimic any kind of liver disease in childhood other than neonatal cholestasis, the diagnosis rests upon laboratory findings. Ensure that the lab you are relying upon participates in an external quality assurance scheme. Each of the tests has limitations, and particular tests vary in usefulness in different clinical situations.

Plasma Ceruloplasmin

Plasma ceruloplasmin (CP) may be measured immunologically or by enzymatic activity; the latter is more accurate in WD, where apoceruloplasmin may contribute to the level measured immunologically. Be aware that CP is an acute phase reactant, so may be elevated into the normal range in WD with active liver inflammation, is produced in the liver and so may be reduced in cirrhosis of other causes, may be reduced in WD or aceruloplasminemia heterozygotes, may be low in protein-losing enteropathy, and is a glycoprotein and so may be reduced in some disorders of glycosylation. Twenty percent of WD patients may present with normal ceruloplasmin levels. Values exceeding 30 mg/dl are rare in WD [2].

24-H Urinary Copper Excretion

Accuracy of 24-h urinary copper estimations depends on the collection, the container, and the lab. Values greater than 40 micrograms/24 h raise the suspicion of WD; values greater than 100 micrograms/24 h make it very likely.

In the originally described penicillamine challenge test using a cutoff value of 25 mcmol/24 h (1600 mcg/24 h), the test was abnormal in 15 of 17 Wilsonian patients with active disease and 1 of 58 non-Wilsonian patients. The test was again evaluated by Müller et al. who showed sensitivity to be 76% and specificity 93% in the whole cohort of patients. However, and most importantly, the sensitivity was as high as 92% in symptomatic patients and only 46% in asymptomatic patients [20]. Others have found a cutoff value five times the upper limit of normal gives good differentiation. The test has not been evaluated in adult neurologically presenting cases.

Serum Copper

Serum copper is largely CP bound, so it will be low in mild or presymptomatic disease but sometimes raised (e.g., in acute liver failure) if serum-"free" copper is raised. Calculated free serum copper according to the formula [total copper – 0.3% ceruloplasmin] is in practice a disappointingly inaccurate parameter, but the recently described direct measurement of "relatively exchangeable copper" holds more promise [21]. Other methods to measure non-exchangeable copper are also being developed.

Liver Copper

When a liver biopsy is performed in suspected WD patients, a specimen for measurement of liver copper should always be obtained. Values equal to or higher than 250 μg/gm of dry weight are considered to be typical for Wilson's disease with. In chronic cholestasis conditions, the liver copper content will also be elevated, but this should not be a source of diagnostic confusion. Values less than 250 micrograms/g may be found in WD cirrhosis, where the centers of large

regenerating nodules and tracts of fibrous tissue will both have lower Cu concentrations. Thus, Ferenci et al. assessed the hepatic copper content of 106 patients at the time of diagnosis of Wilson's disease of whom 19 patients had a liver copper concentration below 250. Values equal to or higher than 250 μg/gm of dry weight are considered to be typical for Wilson's disease. In chronic cholestasis weight has a poor sensitivity (82%) and very good specificity. The low range (50 μg/g dry weight) has a higher sensitivity, but lower specificity as well as a positive predictive val [22]. The most comprehensive study analyzed liver samples from 691 patients with various liver diseases, including 178 with WD. Mean liver copper content was significantly higher in patients with WD with liver dysfunction than in asymptomatic patients or patients with neurological dysfunction without signs of liver disease. All patients with WD with liver dysfunction had liver copper levels above 250 mg/g dry weight, but a high proportion (47.8%) of patients with primary biliary cirrhosis or primary sclerosing cholangitis also had liver copper values _ >250 mg/g dry weight [23].

Liver biopsy is rarely performed in adult neurological patients, so the value of liver copper quantification in neurological presentation is not established.

The data from children with WD is scarce. Nicastro et al. reported liver copper >250 μg/g dry weight in 28 of 30 WD children with mild liver. Among WD patients, 2 children (7%) had liver copper level <75 μg/g dry weight, while 4 (6%) of 24 controls had liver copper levels >50 μg/g dry weight [24].

Testing Strategy

Mutations in the Wilson's Disease *ATP7B* Gene, Locus 13q14.3, and Genetic

The WD gene comprises 80,000 base pairs on 21 protein-coding exons and a poorly characterized promoter. As described earlier the Human Gene Mutation Database references 858 mutations, including 539 missense/nonsense, 71 splicing, 16 regulatory, and 146 small deletions. Therefore, genetic testing is challenging but is crucial in making or confirming diagnosis.

Mutation detection by direct sequencing is now widely available, but bear in mind the following. First the testing strategy should be appropriate to the population served. For heterogeneous populations, it may be known which exons have the highest mutation frequency. For the UK, these are exons 14 (24%), 8 (20%), and 2 (12%). Analysis of these three exons would detect 56% of mutations, while analysis of exons 2, 5, 8, 13, 14, 18, and 1920 would detect 82% of the mutations in this population. Second, some patients have been found to have three mutations, two on one chromosome ("in cis"). Therefore, finding two mutations does not necessarily mean the patient has WD; they might be in cis so that he/she is actually a carrier, i.e., has one normal allele on the other chromosome. This mistake is avoided by proving that one of each of the patient's mutations is present in each parent. Third, though large deletions are rare, they can occur and may be missed, and the patient may be wrongly described as just having the one point mutation on the other chromosome. Fourth, apparent parent-to-child transmission of WD occurs, the unaffected parent turning out to be. Therefore, family screening must extend to the children of known parents with WD. Mutation testing for WD should be restricted to laboratories which participate in the European Molecular Quality Network.

Next-generation sequencing (NGS) is very efficient and can identify both mutant alleles in 95% of affected subjects, but there are several pitfalls as indicated earlier. There is also a risk of identifying variants of unknown significance (VUS) which pose diagnostic difficulties, and with the new cell models, molecular characterization of novel ATP7B variants may help to functionally categorize the mutation and to assist in early WD diagnosis [25].

Diagnosis in Different Clinical Scenarios

In central Europe, a rapid H1069Q test may be the most cost-effective first-line test in any suspected WD patient. Otherwise, one is reliant on biochemistry. Tailor your testing to the clinical scenario. In acute liver failure, suspect WD if there is Coombs negative hemolysis, a high bilirubin (>300micromol/l), and relatively low transaminases (100–500 IU/l) and alkaline phosphatase (<600 IU/l) and alkaline phosphatase IU/l/total bilirubin mg/dl ratio <2. Urine copper and a penicillamine challenge are probably the most valuable copper tests. In active but non-fulminant disease, the penicillamine challenge test is useful, whereas in presymptomatic siblings, it will be useless, but ceruloplasmin should be discriminatory and there is of course time to get a mutational analysis.

WD can present as early as 2 years of age, and early presentation can be challenging to establish diagnosis. Biochemical tests including urinary copper excretion may be less sensitive in very young children; therefore, the diagnoses usually require a combination of tests. If molecular tests are inconclusive, liver copper content should be measured [26].

Making diagnosis in infants is also difficult. Healthy neonates and young infants present with naturally low levels of

ceruloplasmin, and liver copper is increased in infancy, so these tests cannot be used directly for making diagnosis of Wilson's disease.

However, the value of biochemical tests for making early diagnosis should not be completely excluded. Early screening for Wilson's disease may be possible if new cutoff values for ceruloplasmin are established as indicated by Kroll and coworkers who analyzed blood spots for ceruloplasmin concentration from 1045 anonymous newborn screening specimens and from 2 Wilsonian patients. Ceruloplasmin levels were extremely low in Wilsonian patients (2.8 and 2.6 mg/dl) compared to healthy infants (6.5 to >60 mg/dl) [27].

Scoring System for Diagnosis of Wilson's Disease

As making diagnosis of Wilson's disease based on clinical symptoms or single biochemical test is difficult, several parameters need to be taken into account all together. In 2001 a scoring system was proposed and further evaluated by Ferenci et al. [28].

The patients with a total score of at least four are diagnosed to have Wilson's disease. The patients with a total score of two to three are considered as "likely to have Wilson's disease, yet more investigations had to be performed." The diagnosis of Wilson's disease was judged to be improbable for scores between zero and one [29].

In order to test this scoring system, Dhawan et al. investigated records of 143 children with chronic liver disease, aged at least 5 years. Among the patients studied, 53 children were diagnosed to have Wilson's disease (median observation time 11 years), and 90 children had other liver diseases. Fifty patients with Wilson's disease had a score >= 4 (true positives). A total of 85 true negatives with a score of either 2–3 (40 children) or <1 (45 children) were observed. Both sensitivity and specificity of this scoring system were higher than 94% [30].

Genotype-Phenotype

It would be prognostically useful if there were a clear mutation/phenotype relationship, but there is not.

It is common to find differing phenotypes among siblings even for identical twins. Two statements may be made. First, patients homozygous for H1069Q tend to present later and with neurological disease. Second, there is a suggestion that fulminant hepatic failure is more likely in patients with truncating mutations [31–33].

Gene Modifiers

Searching for modifier genes differs from searching for disease-causing genes. In searching for genes involved in the disease, individuals are usually defined as affected or unaffected, but in searching for modifier genes, the affected individuals are related to the clinical symptom variability. The modifier gene effect can vary from strong effects, similar to those observed in a monogenic disease, to much milder effects typical for multifactorial, complex diseases. The strong effect relates mostly to highly penetrant mutations, whereas mild-to-moderate effects relate to single nucleotide polymorphisms (SNPs).

Rare allelic variants in ESD and INO80 seem to associate with a neurological phenotype while rare variants in APOE and MBD6 with age of onset [34].

The *HSD17B13*:TA allele modulates the phenotype and outcome of WD. This allele likely ameliorates hepatic fibrosis and reduces the transition from copper-induced hemolysis to fulminant disease in patients with WD [35].

Treatment

The aim of therapy is to decrease copper absorption and accumulation in the liver and central nervous system. For all WD patients, lifelong medical therapy is required. Conventional medical therapies are based on either copper chelators (penicillamine, trientine, tetrathiomolybdate) or zinc. Chelators mobilize intracellular copper into the circulation which is then excreted with the urine. Zinc decreases copper content mainly by reducing intestinal absorption. It induces copper-binding metallothionein both in enterocytes and hepatocytes, reducing the damaging effects of free liver copper.

Definite treatment is liver transplantation which is however indicated only in selected cases where rapidly progressing liver failure cannot be reversed by medical therapy.

Evidence from observational studies shows very good long-term prognosis on medical therapy with a survival comparable to the general population, even if some of them do not normalize completely aminotransferase activity [36, 37]. Poor compliance is regarded to be the major problem and the major reason of poor outcome.

Copper Chelators

Penicillamine and trientine are chelating agents regarded to be the first-line therapy in severe liver disease, but also effectively used in neurological symptoms and in asymptomatic cases. The major limitations for their use (mainly penicilla-

mine) are side effects and toxic reactions reported in almost 10% of adult patients. The major risk of chelating therapy is worsening of neurological symptoms (in neurological presentation) after therapy is started, and for this reason the doses are usually increased gradually (within a few weeks). Penicillamine therapy is more common in Europe (according to data from Eurowilson project – unpublished). Trientine can replace penicillamine in patients presenting with penicillamine intolerance (trientine is regarded to be safer), but sometimes it is used as a first-line therapy. There have been only rare reports of allergic reactions, arthralgias, muscle cramps, and sideroblastic anemia induced by trientine. Similar efficacy of trientine compared to D-penicillamine was shown in one large study in WD adults with liver symptoms, but trientine was also associated with a higher risk of neurologic worsening of symptomatic neurological patients compared to D-penicillamine [38]. In the pediatric study of trientine as a second-line therapy in 16 children with D-penicillamine intolerance or adverse events, liver function normalized in the majority of children, but trientine did not improve accompanying neurological or psychiatric symptoms [39].

Tetrathiomolybdate is another copper chelator which was mainly investigated in neurological presentation of the disease. It seems to be a more powerful decoppering agent than trientine and penicillamine, but there is limited experience in pediatric practice. According to Brewer et al., it showed very strong control of free copper levels over the 8 weeks of treatment in adults, and it was better than trientine in the tetrathiomolybdate/trientine double-blind study [40].

The full dose of penicillamine is 20 mg/kg/day – given in three doses. Adults receive 1 g per day up to 2 g if there is a poor response. Side effects include hypersensitivity reactions (usually transient and at the beginning of therapy), proteinuria, fever, lymphadenopathy, and bone marrow suppression. Patients should be monitored for side effects with blood count, urine analysis, as well as liver and renal tests. In children, the dose of D-penicillamine is also usually increased progressively to 20 mg/kg/day given in two or three doses, even in patients with only liver presentation. The adverse events like hypersensitivity and proteinuria and hematologic toxicity can be observed and require immediate discontinuation and switching to trientine or zinc salts [41].

The recommended dose of trientine in children is up to 20 mg/kg/day in 2–3 divided doses. The drug is best given 1 hour before or 2–3 hours after food for optimal absorption (Table 68.1). Trientine tablets either must be kept refrigerated (2-HCL trientine) or do not require any special storage conditions (4-HCL trientine).

Zinc

Zinc can be alternatively given to the patients with Wilson's disease, but it is not recommended in severe liver disease with abnormal synthetic function as chelating agents are believed to be more effective in producing negative copper balance. Zinc seems to be safe, and it was the reason for its preferential or alternative use in neurological and asymptomatic cases. It is not associated with neurological deterioration which could be the main reason for its common use in neurological patients. Zinc is also used for maintenance therapy after the induction phase with chelators.

Zinc is given in the dose of 25 mg of elemental zinc three times in younger children (<50 kg) a day or 50 mg three times a day in older children. In very young children under 5 years of age, the dose is not well defined. Patients should be fasted, so zinc should not be given between meals.

There are two different chemical zinc preparations used – zinc sulfate and zinc acetate. Zinc acetate is more commonly used and has a better tolerance profile but is also more expensive. Zinc sulfate is also commonly used but may cause more significant side effects – like nausea, vomiting and epigastric pain, mild gastric irritation, decreased blood iron levels, and anemia. We have also recently reported gastric/duodenal mucosal ulceration or erosion [42]. These side effects may lead to discontinuation of the therapy, and it could be the reason for slightly elevated transaminases observed on this treatment.

Different Clinical Presentations and Choice of Medical Therapy

The choice of medical therapy is related to clinical presentation of Wilson's disease, and there is an agreement to use chelators for acute liver failure as first-line treatment. However, there are still many controversies on preferential use of chelators or zinc for other clinical situations. In severe liver insufficiency due to Wilson's disease, penicillamine or trientine therapy should be started immediately after diagnosis is made. Improvement does not appear soon after therapy was started, and usually first after one or several months, liver function tests improve. For other hepatic presentations of Wilson's disease, chelators seem to be more effective as indicated by the review of 288 patients with a median follow-up time of 17.1 years presented by Weiss et al. who showed that increase in activity of liver enzymes occurred more frequently from zinc therapy (14/88 treatments) than from chelator therapy (4/313 treatments; $P < 0.001$). Similarly, the

Table 68.1 Pharmacotherapy in Wilson's disease

Drug	Dose	Comment
D-penicillamine	20–35 mg/kg/day	
Trientine preparations		
2-HCL 250 mg capsules	500–750 mg in 2–3 doses	Keep in refrigerator Give before or 2h after meals
2-HCL 300 mg capsules	1.2–2.4 grams (4–8 capsules) daily 2–doses	Store in refrigerator
4-HCL 300 mg tablets	450–975 mg (3–6.5 tablets) in 2–4 dos	This medicinal product does not require any special storage conditions
Zinc acetate/sulfate	Up to 5y: 2×25 mg[a]or less 6–16 y: 3×25 mg[a] >16y: 3×50 mg[a]	1h before or2h after meals

[a] Elemental zinc

survival without transplantation was better for chelating agents. Patients who did not respond to zinc therapy showed hepatic improvement after reintroduction of a chelating agent [29].

Zinc is commonly used in neurological presentation where it seems to be as effective as chelators [43].

Still, it is difficult to compare both therapies as most of the evidence comes from observational trials. Wiggelinkhuizen et al. performed a systematic review of zinc and penicillamine therapies for Wilson's disease. They found 1 randomized controlled trial and 12 observational trials. Patients with liver presentation seemed to respond better to penicillamine. Zinc appeared to be better option in presymptomatic and neurological cases [44].

It is also difficult to compare trientine and penicillamine; however, trientine appears to be a safer option, but due to high costs, its use is limited in many countries.

For asymptomatic cases it is not clear at what age therapy should be started, but there is an agreement not to start in the first year of life.

Treatment Monitoring

Compliance with treatment, efficacy, and safety monitoring are the most important issues in WD treatment.

During the correct treatment of WD patient with chelators, the recovery of liver function tests (LFT) and clinical symptoms of liver disease (e.g., jaundice, ascites, coagulopathy, etc.) are expected during the first 2–6 months and should continue through the first year of treatment. Zinc may require longer time. In case of neurological symptoms, the expected improvement takes longer time, is slower, and can be observed up to 3 years after treatment start.

Adequacy of D-penicillamine treatment could be measured with daily urinary copper excretion, non-ceruloplasmin-bound copper (NCBC) in serum analysis, as well as test of urinary copper excretion at baseline and 48 hours after D-penicillamine cessation or patient's drug intake diaries, but all these methods were not properly validated. Urinary copper excretion after D-penicillamine introduction shows value in excess of 1000 μg/24 hours, but it settles to to 300–500 μg/24 hours after 1 year of correct treatment. The results below 300 μg/24 hours suggest overtreatment or noncompliance with treatment and need to be immediately explained (decrease the D-penicillamine dose or ask the patient to correct drug intake). Serum NCBC levels (normal range: 5–15 μg/dl) may further help with adequacy of WD treatment assessment but may be not reliable. The results <5 μg/dl suggest overtreatment and copper deficiency and >15 μg/dl lack of compliance with WD treatment. Urinary copper analysis before and after 48 hours of cessation of D-penicillamine could be helpful in treatment monitoring. In patients with effective WD treatment, urinary copper excretion of under 50 μg/24 hours is usually expected after 48 hours of D-penicillamine cessation.

For safety reasons WD patient treated with D-penicillamine should also undergo analysis of liver tests, complete blood count, and urine analysis at least twice annually. 24-hour urinary copper excretion should be performed at least annually [45].

Monitoring of zinc therapy is based on measuring serum zinc levels, 24-hour urinary copper, and zinc excretion. Brewer et al. advise to test zinc serum and urine copper levels during therapy. A zinc serum level less than 125 μg/dL generally indicates poor compliance. Urine copper levels should not exceed 50–75 μg/24 hours [2].

Diet in Therapy of Wilson's Disease

The role of the avoidance of copper-rich food products (nuts, chocolate, liver, shellfish) is not well established, but they are traditionally excluded from the diet. Avoiding copper-rich food (shellfish, nuts, chocolate, mushrooms, and organ meats) is advised until remission of symptoms and biochemical abnormalities.

Liver Transplantation

Liver transplantation is indicated for acute liver failure with fatal prognosis. Acute liver failure with encephalopathy does not respond to medical therapy and requires immediate listing for liver transplantation. The indications for liver transplantation in acute liver failure without encephalopathy are less clear as many patients recover with chelation therapy. It is advised to use the scoring system developed at King's College Hospital, which should be applied daily. A score >=11 indicates the need to list urgently for liver transplantation. A deteriorating score similarly raises the need to list. A stable or improving score is an indication to continue with medical therapy [34].

The indications for liver transplantation are less clear in patients with predominantly neurological symptoms. In general neurological symptoms are not an indication for liver transplantation, but there are some reports of neurological improvement after liver transplantation (however some patients deteriorated).

Therapy in Pregnancy and during Lactation

Treatment should be continued throughout the pregnancy to avoid exacerbation of symptoms. Zinc is commonly used in this period as it appears to be safer, but according to some practices, penicillamine can be used in decreased doses even if safety of this therapy is not well proven. The possible teratogenic effects of D-penicillamine and the influence on wound healing (cesarean section) should be considered, and the dose should be decreased about 25% only during the first trimester (the higher ratio of teratogenic effect possibility). Moreover, all current drugs used in WD pass into the milk, that's why the children of WD mothers treated with anti-copper agents shouldn't be fed by breast (to avoid the copper deficiency syndrome as well as other adverse drug reactions).

Novel Therapies

The present medical therapy is effective but not enough in some clinical situations like acute liver failure and significant neurological impairment. Therefore, new drugs are being tested like bis-choline tetrathiomolybdate [46]. Recent progress in gene therapy may be also used for treatment of Wilson's disease.

Compliance

Lifelong compliance to drug therapy seems to be a major problem in Wilson's disease which decides about prognosis. Stopping medication may lead to severe organ damage, or even death, within a time period that can be as short as a couple of months. There are several reasons for poor compliance: nausea on zinc therapy, fear for numerous side effects of penicillamine, revolting age (teenagers), as well as psychiatric problems seen in Wilsonian patients. Duodenal and gastric ulcerations identified in children under zinc sulfate therapy can explain poor compliance in some patients. Urine copper excretion and free copper concentration are also good indicators of compliance during penicillamine or zinc therapy.

Conclusions

Even if prevalence of Wilson's disease is not high, it should be included into differential diagnosis of chronic liver disease and acute liver failure in children >1y of age. Disease can be diagnosed early in children with increased transaminase activity and/or hepatomegaly. The scoring system for diagnosis of Wilson's disease may be helpful, and it is highly specific. Except for molecular diagnosis, no single test can be used to establish diagnosis or for screening.

It is difficult to compare effectiveness of different therapies, but penicillamine and trientine appear to be drugs of choice for severe liver damage. Zinc is regarded to be safer and is mainly used in mild liver, neurological, or asymptomatic disease.

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Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) was first described in 1980 in obese adults who had a pattern of injury similar to alcoholic hepatitis but who denied alcohol consumption [1]. The disease was subsequently described in children in 1983 [2]. NAFLD is a condition characterised by liver steatosis with or without inflammation and fibrosis, most often in the setting of overweight or obesity. Inflammation implies the diagnosis of nonalcoholic steatohepatitis (NASH) which previously was felt to be a more progressive condition than steatosis without inflammation; however, it is now better understood that inflammation is not a good predictor of disease progression, and the term steatofibrosis may be more applicable [3].

The importance of this disease is borne out by the dramatic increase in its prevalence with an estimated 30% of adults and 10% of children affected in the USA, with similar prevalence in children in the UK and Australia and with a higher prevalence in Central America [4–7].

It is likely that a significant number of those affected by NASH will go onto end-stage liver disease and/or hepatocellular carcinoma within decades [8]. In fact, NAFLD in adults is rapidly becoming the most common indication for liver transplantation and is now the most frequent indication in American women [9]. As NAFLD is likely to continue to increase in prevalence, this will unfortunately put a huge burden on an already overstretched liver transplantation service [4].

This rise in prominence of NAFLD, the liver manifestation of the metabolic syndrome, is not surprising given the close association with obesity. Body mass index does not measure the entire problem however, with reports of patients with 'lean NAFLD' who may not meet criteria for obesity or overweight based on BMI. These patients are most often metabolically unhealthy, with a large waist circumference and other features of the metabolic syndrome. The close association with this phenotype led to the suggestion that NAFLD should be known as metabolically associated steatohepatitis (MASH/MAFLD) [10]. This nomenclature may not be appropriate to apply to children and young people, however, in view of potential confusion with distinct inborn errors of metabolism which may also present as fatty liver [11].

Both genetic predisposition and lifestyle factors influence the development and progression of NAFLD, the pathophysiology of which has not yet been fully elucidated. In view of the startling prevalence of NAFLD and the potential to progress to serious liver disease, the ability to recognise and manage the condition in children is of great importance.

Epidemiology and Predisposing Factors

The increase in prevalence of NAFLD is undoubtedly directly associated with the epidemic rise of obesity. Prevalence of childhood obesity across the globe has increased dramatically over the last three decades. The definition most widely used for obesity in childhood is body mass index (BMI) > 95th percentile and overweight as a BMI between the 85 and 95th centile. The 'normal' BMI varies with age and sex, and different centile charts are available for different populations. More than 370 million children and young people aged 0–19 years are now overweight or obese (http://globalnutritionreport.org) and are likely to remain so in adulthood, and thus more likely to develop NAFLD and type 2 diabetes.

Variation in reports of prevalence of NAFLD is partly due to the different methods of detection and a lack of clarity regarding the definition of the disorder [7, 12, 13]. Liver biopsy is the criterion standard for diagnosis of NAFLD, but clearly this is not feasible as an epidemiological tool and proxy markers such as abnormal transaminases and/or the presence of an echogenic liver on ultrasound are often used

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to define the disorder. The true sensitivity, specificity and predictive value of these proxy markers are unknown, and it is well recognised that an elevation of transaminases may only occur in 60% of cases of fibrotic NAFLD [14]. As the reference range for AST and ALT is derived from population data including those with undiagnosed NAFLD, the use of these markers as a proxy for NAFLD is flawed [4, 15]. In addition, there is considerable variation in the normal ranges for laboratory values across different institutions.

Nevertheless, in the absence of a more robust noninvasive diagnostic test, population studies have used an elevated ALT (in the absence of other diagnoses) as definition of the disorder. In one US-based study, an elevated ALT was found in 8% of 5586 adolescents aged 12–19 years [16]. Park et al. reported a prevalence of 3.2% in 1543 Korean teenagers using ALT >40 [17]. In Japan a population-based study found 2.6% of children to have NAFLD based on ultrasound [18]. In a study from Italy of 268 obese children, 44% had NAFLD using ultrasound and elevated ALT [19]. Studies of liver biopsy findings give a more accurate reflection of prevalence as NAFLD is a histological diagnosis. One of the most useful studies reporting prevalence in the general paediatric population is an autopsy study of 800 children and young people who died of accidental injury. Schwimmer et al. reported fatty liver in 9.3% of this cohort, with NASH present in 3% [5].

The prevalence of NAFLD appears to increase with age, and in general, boys are more at risk [5, 20, 21]. Ethnic variations also exist; Hispanic children and adolescents have a greater risk of NAFLD compared to Caucasian children. Black, non-Hispanic children are less susceptible despite a higher incidence of insulin resistance [5, 21, 22]. This mirrors findings in adults [6, 23, 24]. Both genetic and environmental factors are likely to be involved in ethnic distribution. Familial clustering is also seen [25, 26] with a strong heritability in first-degree relatives [27].

The advent of genome-wide association studies (GWAS) and candidate gene studies has significantly advanced our understanding of genetic susceptibility to NAFLD. Though a smaller body of evidence exists in genetic variation in susceptibility of children with NAFLD than in adults with the condition, children are arguably most likely to demonstrate the effects of genetic variation. We now understand that single nucleotide polymorphisms (SNPs) in DNA (resulting in the altered expression of a gene or altered protein function) and other epigenetic modification influence the phenotype of this polygenic disease [28]. In both GWAS and candidate gene studies, variants in certain genes, namely, patatin-like phospholipase domain-containing protein (PNPLA3; adiponutrin) variant I148M, the transmembrane superfamily 2 (TM6SF2) variant E167K and variants in GCKR (glucokinase regulatory protein) and MBOA7 (membrane-bound O-acyltransferase domain containing 7), amongst others, most consistently convey a susceptibility to NAFLD in both adults and children [29–32].

Perhaps best described and most frequently cited is the variant within PNPLA3, where the mutated protein accumulates on the surface of lipid droplets in the hepatocyte, altering lipid remodelling, and also promotes retinol release prompting inflammation and fibrogenesis [33, 34]. The coexistence of obesity amplifies the effect of the variants [35], and the cumulative risk of genetic variants is also of importance. In a study of 450 children, the combination of variants in *PNPLA3*, *TM6SF2*, *GCKR* and *MBOAT7* explained 19% of hepatic fat fraction (HFF%) variance as quantified by MRI. There is amplification of this effect in the presence of obesity and overweight [36]. Understanding the genetic variation contributing to disease in an individual may allow more targeted prevention and reversal of disease.

Epigenetic factors, particularly microRNA, have been implicated in pathogenesis of NAFLD. Epigenetic modification is unrelated to changes in DNA sequence. MicroRNAs are small soluble RNAs which influence the translation of certain genes. The relative underexpression of microRNA-122 has been described in NAFLD [37].

The importance of the antenatal environment on metabolic programming has been well established since the reports of Barker [38, 39]. Antenatal programming of a child's liver to injury due to lipid accumulation, oxidative stress and innate immune dysfunction may play a role in the susceptibility to NAFLD [40].

In rodent models, the effect of a high-fat diet (HFD) in dams can be seen in the predisposition to developing fatty liver in offspring. A cumulative effect can be seen when the pup offspring of HFD dams are fed with a methionine choline-deficient (MCD) diet, a well-established model for NAFLD [41]. An alteration of DNA methylation and a decrease of microbiome diversity in the gut were found possibly to mediate the effect.

In mice, maternal obesity and a postweaning high-fat diet were independent risk factors for steatosis and steatohepatitis and fibrosis at 12 months with a significant increase in liver injury when both risk factors were present [40]. A macaque model of HFD prior to breeding and during pregnancy showed similar findings with increased steatosis in the offspring of treated mothers [42].

The equivalent antenatal priming of the liver in humans, which may then be exposed to decades of excess nutrition and sedentary behaviour, may result in a more severe phenotype or progressive disease.

The early effects of maternal metabolic control on the infant liver have been investigated. Stillborn infants of mothers with gestational diabetes mellitus (GDM) were found to have steatotic livers in 78.8% of cases versus 17% of those born to nondiabetic mothers [43]. Live-born infants to mothers with GDM in another study demonstrated a higher

liver fat content on MRI in those born to mothers with GDM than controls [44]. As adipose tissue deposition does not occur until the third trimester, there is foetal hepatic accumulation of excess substrate together prior to this point with an increase in de novo lipogenesis due to a high transplacental glucose supply in infants of diabetic mothers [38]. This is in the context that up to 60% mothers are now obese at the time of conception and thus at high risk of GDM [45].

Further evidence of the importance of a healthy pregnancy is that birth weight is associated with the development of NAFLD in childhood in a large cohort [46].

Early nutrition is likely also important in modification of the risk of later NAFLD. Nobili and colleagues have studied breast feeding habits in a cohort of children with NAFLD and concluded that breast feeding is protective against progression of the disease from simple steatosis to steatohepatitis and fibrosis [47].

The role of maternal obesity and the method of delivery and of early infant feeding may all be mediated in part via the microbiome and a decrease in diversity conveyed to the infant microbiome which is associated with later obesity. Soderberg et al. demonstrated this effect in mice by colonising germ-free mice with microbiota from 2-week-old pups born to obese mothers or to normal weight mothers. Mice colonised with the microbiota from infants of obese mothers demonstrated increased liver injury with a histological pattern similar to paediatric patients with NAFLD [48].

Nutrition and physical activity are critically important environmental factors determining risk of NAFLD, with lifestyle modification as the primary recommendation in the prevention and management of the disease [49, 50]. Excess food intake and lack of exercise contribute to weight gain and contribute to the progression of liver fibrosis and inflammation in patients with NAFLD [51, 52].

Specific dietary factors either protect against or exacerbate the development and progression of NAFLD. Food-based analyses have suggested that higher meat and fructose [53–55] and higher consumption of low-nutrient, high-calorie, high-salt food [56] are associated with NAFLD. Fructose has been identified as a particular culprit in increasing fat, inflammation and fibrosis [57, 58]. Fewer paediatric studies of dietary composition have been undertaken in NAFLD; however, a large study from Australia compared Western/health diet in 993 14-year-olds to later development of NAFLD on ultrasound and found a significant association of Western diet and development of steatosis at 17 years [59]. A study in 82 obese Greek children revealed that a diet higher in carbohydrates and saturated fatty acids and lower in omega-3 was associated with NAFLD [60].

Dietary chemical composition of fatty acids may be an important factor in lipotoxicity observed in insulin resistance. Palmitic acid rather than oleic acid results in lower steatosis but in higher cell death and impaired insulin signalling [61]. A study of fish intake and omega-3 fatty acid intake in children with NAFLD revealed a dietary deficiency of both was associated with increased portal and lobular inflammation [62].

Vos et al. described an association of dietary vitamin E insufficiency and increased steatosis in children with NAFLD [63].

Pathophysiology

NAFLD can be thought of as the hepatic manifestation of the metabolic syndrome (linking obesity, insulin resistance, hypertension and hyperlipidaemia). The pathogenesis of the condition is still incompletely understood.

Insulin resistance (IR) is a finding in up to 80% of children with NAFLD and has a similarly high prevalence in adults with the condition [22, 24, 64, 65]. It is widely accepted that IR and the resulting hyperinsulinemia seem to play a major role in the development of hepatic steatosis and steatohepatitis. The molecular mechanism leading to the involvement of IR in the development of NAFLD is complex however, and has not yet been fully elucidated.

The 'two-hit hypothesis' proposed in 1998 consisting of a first hit of liver fat accumulation followed by a trigger for inflammation and fibrosis, was first thought of as a model of liver injury in NAFLD. We now know there are likely multiple 'hits' including oxidative stress, intestinal dysbiosis, bile acid dysregulation [66, 67] and adipocytokines from a high visceral fat mass or saturated free fatty acids (FFA) [68–70]. Figure 69.1 demonstrates the interplay of the multiple hits.

Steatosis

Macrovesicular steatosis is characterised by the accumulation of triglycerides (formed of glycerol esterified to three fatty acids) in the hepatocyte. Steatosis is conventionally thought to arise from increased hepatic supply of FFA as a result of obesity and associated extrahepatic insulin resistance.

Normally adipocytes store fat after meals and release fat during fasting by lipolysis. In the liver, carbohydrate is stored as glycogen, and when the liver is saturated, de novo lipogenesis occurs via acetyl coenzyme A and fatty acid synthetase. The third source of fatty acids as a substrate for the liver is dietary.

In the setting of normal insulin sensitivity, fatty acids undergo esterification to triglycerides in the hepatocyte and are then exported from the cell as very low-density lipoproteins (VLDL) via apolipoprotein B enzyme activity

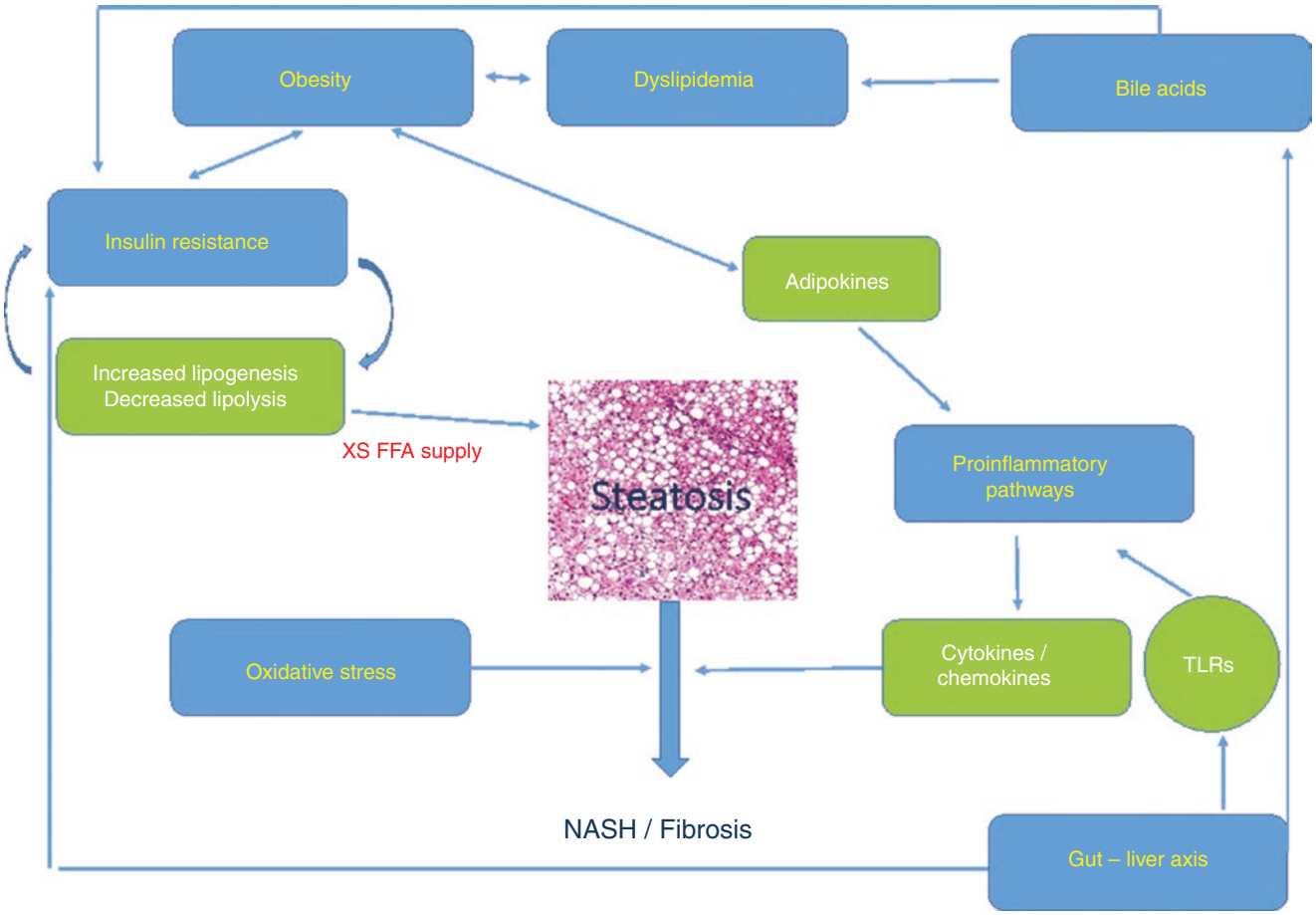

Fig. 69.1 Interplay of multiple hits involved in the pathogenesis NAFLD

[71]. Alternatively they may undergo β-oxidation in the mitochondria or oxidation in the peroxisomes or microsomes. Uptake of FFA into the mitochondria requires carnitine palmitoyl acyl-transferase which is inhibited by insulin and malonyl coenzyme A.

The net retention of lipids is the primary problem in steatosis. The most consistent predisposing factor to hepatic fatty acid accumulation is insulin resistance, though other factors may be involved.

In normal physiological circumstances, the role of insulin includes glycogen synthesis, glycolysis and protein and lipid synthesis. In the postprandial state, insulin promotes lipogenesis and suppresses lipolysis and gluconeogenesis. The normal fall in insulin with the fasting state, which is accompanied by an increase in glucagon and catecholamines, mediates glycogenolysis and gluconeogenesis. These processes are accompanied by lipolysis and increased lipid oxidation. Sensitivity to insulin is increased by adiponectin and decreased by TNFα [72].

In the setting of insulin resistance, fat-laden and insulin-resistant adipocytes continue to release glycerol and FFA into the circulation, and deliver increased free FFA to the liver [73–75]. This in itself may then induce hepatic IR [76]. Hyperinsulinemia and hyperglycaemia promote de novo lipogenesis via upregulation of the transcription factors sterol regulatory element-binding protein 1c (SREBP1c) and peroxisome proliferator-activated receptor γ (PPARγ) [73]. In addition, insulin increases malonyl coenzyme A (an intermediate of FA synthesis) and inhibits carnitine palmitoyl transferase, thereby inhibiting the passage of long-chain fatty acids (LCFA) into mitochondria for β-oxidation [77]. SREBP1c can also be upregulated by glucose and saturated fats, whereas polyunsaturated fatty acids (PUFAs) lead to decreased expression [78]. Increased glucose levels also stimulate lipogenesis through the activation of carbohydrate response element-binding protein (ChREBP), a transcription factor activating the expression of key enzymes of glycolysis and lipogenesis [79, 80].

Hyperinsulinemia also results in decreased triglyceride secretion as VLDL by lowering apolipoprotein B synthesis and stability [81, 82]. Hence, hepatic FFA uptake and lipogenesis outweigh FA oxidation and triglyceride secretion leading to hepatic fat accumulation [83]. In the setting of peripheral insulin resistance, some hepatic insulin sensitivity may be preserved with continuing de novo lipogenesis as a consequence. This is mixed and thought to be medicated

through a functioning insulin receptor substrate 1 (IRS-1) which blocks lipid oxidation, but aberrant IRS-2 serine phosphorylation which fails to suppress gluconeogenesis [71].

Oxidative Stress

Mitochondrial FA oxidation and ketogenesis are increased, and the transcription factor PPARα is activated as a result of fatty acid accumulation [84]. This results in reactive oxygen species (ROS) which lead to oxidative stress and lipid peroxidation. Cell membranes are damaged, and cytochrome c is released from the mitochondrial intermembrane space. In turn, this leads to an imbalance in the flow of electrons over the respiratory chain (RC) creating overreduction of RC complexes which can react with oxygen to form further ROS [85]. ROS may then propagate liver inflammation and injury.

Mitochondrial function has been shown to be impaired in patients with severe steatosis and steatohepatitis [86]. Ultrastructural abnormalities of mitochondria have been demonstrated in patients with NASH [87, 88]. It is not clear if this is a primary or secondary phenomenon however. Mitochondrial abnormalities could be a pre-existing condition enabling the excessive production of ROS in the setting of enhanced FFA ß-oxidation [87]. This could explain why for the same amount of obesity, or for the same degree of IR, certain patients just have steatosis, whilst others develop NASH and cirrhosis. Genetic polymorphisms could also at least partially explain this difference in susceptibility as some could favour mitochondrial dysfunction [89]. Alternatively the overload of the mitochondrial RC, the resulting formation of ROS and subsequent lipid peroxidation products may give rise to mitochondrial damage. There is an inverse correlation of peripheral TNFα levels and measures of insulin resistance with RC enzyme levels suggesting that insulin resistance and cytokine activity may be important in impairment of the mitochondrial RC [90]. Enhanced ROS formation in the vulnerable steatotic liver subsequently triggers lipid peroxidation and the formation of reactive aldehydes such as 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). These give rise to further mitochondrial damage and ROS formation, resulting in a vicious cycle [85]. Hepatic stellate cells may be activated by these molecules, thus leading to fibrosis [91].

Cytokines and Inflammation

Much of the progression from simple steatosis to steatohepatitis is characterised by an inflammatory response [92]. It is clear from both rodent and human studies that hepatic steatosis is associated with a state of chronic inflammation [93–95]. More specifically, hepatic steatosis in this context is associated with nuclear factor κB (NFκB) activation. FFA can directly activate the pathway via a lysosomal cathepsin B-dependent mechanism [96], as can mitochondrial and ER stress [94, 97]. NFκB is a sequence-specific transcription factor that functions as a pro-inflammatory master switch during inflammation. It upregulates the transcription of a wide range of inflammatory mediators including TNFα, IL6 and IL1β. Increased production of inflammatory cytokines by hepatocytes leads to Kupffer cell activation with subsequent inflammatory mediator release and hepatic and systemic insulin resistance [98].

Animal studies have shown that translocation of bacteria from the gut to the liver via the mesenteric circulation can activate Kupffer cells (via CD14/Toll-like receptor 4 (TLR4) binding) and induce a local and systemic inflammatory response [99]. There has been a great deal of interest in gut microbiota and the innate immune response in the context of obesity and insulin resistance [100]. There is evidence that intestinal bacterial overgrowth exacerbates NAFLD and that the prevalence of bacterial overgrowth is higher in those who are obese [101], the portal circulation providing a direct route from the gut to the liver. Manipulation of gut microbiota and elimination of intestinal bacterial overgrowth may thus be promising ways to halt the progression of steatosis to steatohepatitis and fibrosis.

Finally, visceral fat is a highly inflammatory tissue and the source of many inflammatory mediators known as adipocytokines which have an important role in insulin resistance and, most likely, in NAFLD [102, 103]. These adipocytokines, including leptin, adiponectin, TNFα and IL6, are polypeptides produced by both adipocytes and macrophages which infiltrate adipose tissue [104]. Adipokines are involved in the various injury patterns in NASH such as cell death, inflammation and fibrosis [85].

Leptin is a 16 kDa protein, a product of the ob gene, and has important roles in appetite suppression and regulation of energy metabolism [105], with high levels in obese individuals though this is thought to be a result of leptin resistance. The role of leptin in NAFLD is not yet clear though it is thought to contribute as a pro-inflammatory, profibrogenic mediator [106, 107].

Adiponectin is a polypeptide adipokine with a collagen-like domain and globular domain produced in white adipose tissue. It has an important role in insulin sensitivity; as part of their action, thiazolidinediones are known to increase levels of adiponectin [108]. It is also hepatoprotective with anti-inflammatory and anti-fibrogenic properties [109–111].

Hepatocyte Apoptosis

Hepatocyte apoptosis is recognised as an important event in the development of chronic liver disease and has particular prominence in NAFLD [112]. The initiating event in apoptosis may be extrinsically mediated hepatocyte injury (e.g. in autoimmune liver disease, viral hepatitis and ischaemia per-

fusion injury). This is usually directed through pathways involving Fas ligand, TNFα, and TRAIL. Alternatively, intrinsic injury and death may occur via organelle dysfunction when cells are subjected to excessive oxidative stress (ER or mitochondrial), e.g. with drugs/toxins, fatty acids, and iron. This results in altered membrane permeability and RNA damage with cytochrome C release [113]. The injurious mechanism in NAFLD/NASH appears to be due to a combination of extrinsic and intrinsic insults [114, 115].

Though apoptosis is classically thought to be a silent event without provoking an inflammatory response, this is not the case in the liver [114]. Apoptotic bodies can activate stellate cells and Kupffer cells inducing an inflammatory response and leading to the progression of steatohepatitis and fibrosis [116, 117].

Fibrosis

The final common pathway of inflammation, oxidative stress and hepatocellular damage is the development and progression of fibrosis in NAFLD. The process of fibrosis involves the deposition of extracellular matrix within the parenchyma. Cirrhosis, the end stage of the fibrotic process, is characterised by septae and nodule formation. Several different injurious processes will result in fibrosis. Hepatocyte injury, inflammation, apoptosis and death initiate the process which involves a cascade of inflammatory cells, the release of cytokines and the activation of fibrogenic effector cells (mainly stellate cells) [118].

Thus, a number of different processes and mechanisms are involved in the progression of steatosis to NASH: oxidative stress, inflammation, apoptosis and fibrosis (Fig. 69.2). The exact sequence of development of obesity, fatty liver and NAFLD remains unclear. Whether IR causes hepatic steatosis or whether the accumulation of fat in the liver is the primary event leading to hepatic and peripheral IR is also yet to be elucidated [119].

Diagnosis and Histology

Children with NAFLD are often asymptomatic or may present with vague nonspecific symptoms such as abdominal pain and fatigue. The majority are overweight (gender- and age-specific BMI >85th centile) or obese (>95th centile) [120]. Hepatomegaly may be present, and acanthosis nigricans (a black pigmentation of the skinfolds, axillae and

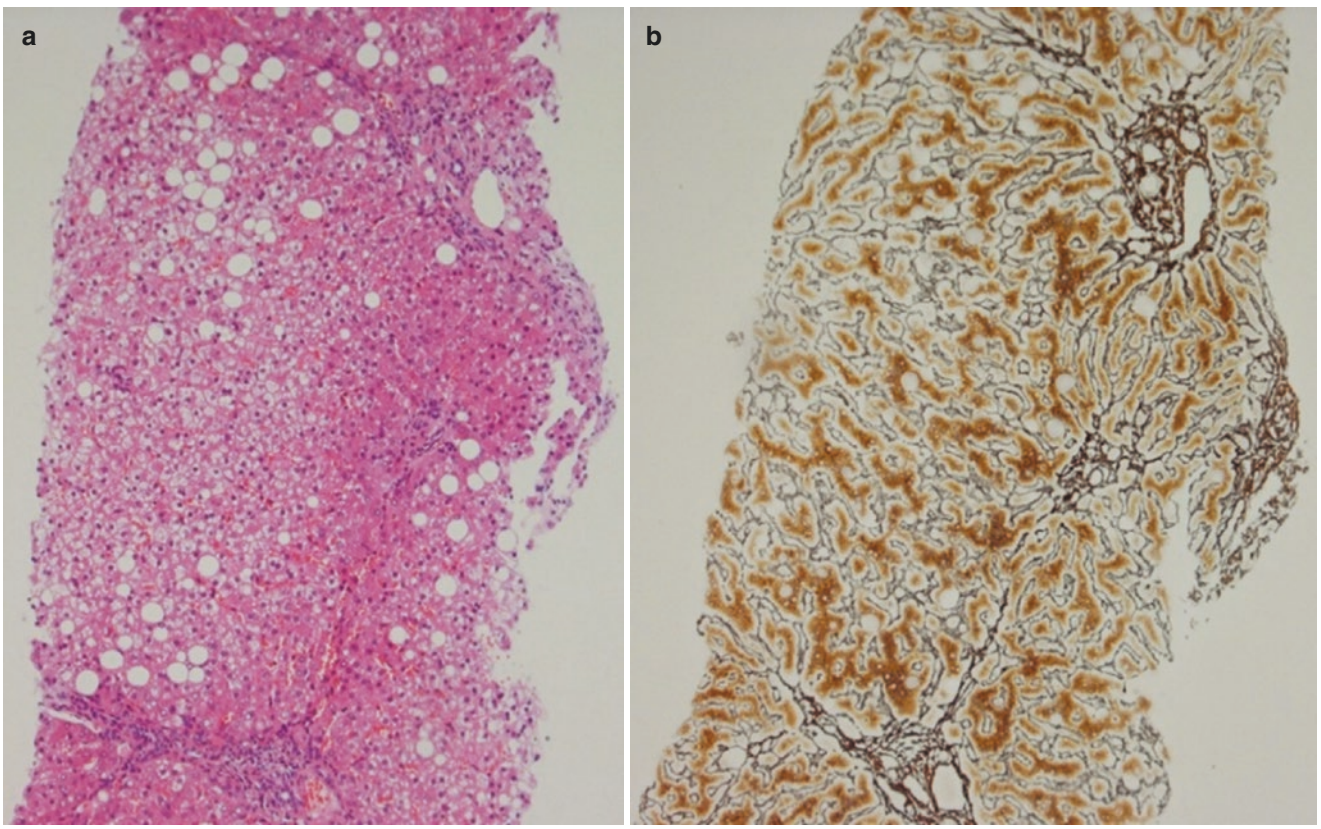

Fig. 69.2 Histology of NAFLD. (**a**) Haematoxylin and eosin staining showing steatosis, inflammation and ballooning. (**b**) Reticulin stain showing fibrosis

neck), often seen in children with insulin resistance (IR), is found in 30–50% of children with NAFLD [22, 121]. The majority of children with NAFLD have insulin resistance as measured by homeostasis model assessment-insulin resistance (HOMA-IR: [fasting glucose (mmol/l) x fasting insulin (IU/l)]/22.5) [22]. A normal HOMA-IR (<90th centile) is generally less than 2.5 but varies with sex and pubertal stage [122]. Children will often have a positive family history for the metabolic syndrome [21].

In the diagnostic work-up of NAFLD, alternative causes of chronic liver disease should be excluded including chronic hepatitis B and C infection, Wilson disease, α1-antitrypsin deficiency, autoimmune hepatitis and drug toxicity (including steroids, amiodarone, oestrogens and antiretroviral treatment). Conditions such as cystic fibrosis, malnutrition and parenteral nutrition-associated liver disease may also present with a fatty liver on ultrasound and can be excluded on clinical or biochemical grounds. In addition, mitochondrial/metabolic disease and cholesterol ester storage disease may also look very similar on liver biopsy and need to be considered. Table 69.1 gives differential diagnoses that must be excluded [11].

Table 69.1 Conditions which need to be excluded before a diagnosis of NAFLD is made

Clinical condition	Clinical features	Biochemical/other features which help distinguish from NAFLD
Wilson disease	May present with chronic liver disease, haemolytic anaemia or more rarely neurological disease in childhood. Look for Kayser Fleischer rings	Low serum caeruloplasmin, high urinary copper pre and post penicillamine, high liver copper, genetics may be positive
α1-antitrypsin deficiency		α1-antitrypsin phenotype ZZ (or ZS) more rarely SS
Drugs – Steroids, amiodarone, alcohol, methotrexate, ecstasy, l-asparaginase, vitamin E, valproate, tamoxifen, antiretrovirals	History of drug ingestion	
Cystic fibrosis-associated liver disease	History/examination	Positive sweat test/genetics positive
Malnutrition	Clinical examination	
Coeliac disease	May have failure to thrive	Tissue transglutaminase positive/positive jejunal biopsies
Hepatitis C		Positive serology
Intestinal failure-associated liver disease	Compatible history	
Mitochondrial disease/fatty acid oxidase deficiency	May be history of neurodevelopmental problems or other system involvement	Abnormal respiratory chain enzymes liver/muscle, abnormal acyl-carnitines/skin fibroblast studies, genetics for mitochondrial disease
Lysosomal; Lysosomal acid lipase deficiency, Niemann Pick C		Positive enzymology or genetics
Galactosaemia	Age of presentation	Abnormal Gal-1-PUT result
Fructosaemia	May be history of avoiding sweets	Enzymology on liver biopsy
Glycogen storage disease	Hepatomegaly, may be short, history of fasting hypoglycaemia	Positive enzymology/genetics glycogen not fat on biopsy
Peroxisomal disorders	May be hypotonic/have wide AF, neurological problems	Abnormal very long-chain fatty acids
Mauriac syndrome	History of type 1 diabetes	Glycogen on liver biopsy
Hypobetalipoproteinaemia/abetalipoproteinaemia	Low serum triglycerides, may have history of fat malabsorption, may be FTT	Low or absent Apo1B levels
Lipodystrophies	Examination	Genetics
Shwachman syndrome	FTT pancreatic insufficiency, bony changes, cyclical neutropenia	Genetics
Inborn errors of bile acid synthesis	Cholestasis in infancy, spasticity later on	Urine bile acids, genetics
Organic aciduria	Neonatal encephalopathy, later developmental delay, mild elevation in transaminases, hyperammonaemia	Enzymology, genetics
Myopathic disorders	Myopathy, elevated CPK	Genetics
Hypothyroidism	Other features hypothyroid	TFTs
Congenital disorders of glycosylation	100 subtypes, can be variable presentation, may have hepatocerebral phenotype	Transferrin isoelectric focusing, genetics
Congenital portosystemic shunts	Presence of focal nodular hyperplasia, high ammonia levels	Imaging
Endoplasmic reticulum function disorders, eg Wolcott-Rallison, NBAS	Recurrent ALF	Genetics
Tyrosinaemia	Jaundice, progressive liver disease, coagulopathy	Urinary succinylacetone, genetics

Recommendations for undertaking biopsy in children with suspected NAFLD are made by a consensus document published by the AASLD, ACG and AGA [123]. These are to perform liver biopsy where the diagnosis is unclear and there is a possibility of multiple diagnoses, before starting therapy with potentially hepatotoxic medications or prior to starting children on pharmacological therapy for NASH. An ESPGHAN guideline was published the same year suggesting that liver biopsy should be performed in children with suspected NASH according to the following criteria to exclude other treatable disease, in cases of clinically suspected advanced disease, as part of an interventional protocol or research trial [124].

Tools commonly used in the work-up and diagnosis of NAFLD in children include ALT, AST and imaging techniques (ultrasound, magnetic resonance imaging/spectroscopy). The sensitivity, specificity and predictive values of these techniques are variable [4]. Franzese et al. studied the incidence of liver involvement in 72 obese children using both ultrasound (US) and transaminases [125]. Fifty-three percent of children had a bright liver on US consistent with liver steatosis, whilst only 25% had elevated transaminases. Molleston and colleagues reported histological abnormalities in 91 children with NAFLD and normal or only mildly elevated ALT. Forty-six percent of the group had some fibrosis with 38% mild to moderate fibrosis and 8% bridging fibrosis [126]. Neither US nor transaminases are good discriminators of histological severity [127]. Ultrasound will detect >20–30% steatosis as increased echogenicity, though this is not specific for fat [128]. Magnetic resonance imaging/magnetic resonance spectroscopy (MRI/MRS) is more sensitive and can detect >5% steatosis [129]. However, neither technique can assess presence of inflammation or fibrosis. Schwimmer et al. used MRI PDFF (proton density fat fraction) to diagnose NAFLD with a cutoff of 5% steatosis. The corresponding cutoff levels for ALT in boys were 42 IU/l and 30 IU/L for girls [130].

Liver biopsy remains the gold standard in differentiation of steatosis from steatohepatitis. The diagnosis of NASH is based on a specific pattern of histopathological findings including macrovesicular steatosis, mixed or polymorphonuclear lobular inflammation, ballooning degeneration with Mallory hyaline and a perivenular distribution of fibrosis in adults (type 1 NASH) [131] (Fig. 69.3). Children often have a different pattern of disease with greater degree of steatosis, less prominent ballooning and portal rather than pericentral accentuation of inflammation and fibrosis (type 2 NASH) [132] (Fig. 69.4). Children and young people may demonstrate type 2 NASH or NAFLD which is a more periportal tract-based pattern [133]. Fifty to seventy percent have a type 2 pattern or a crossover between type 1 and type 2 [127, 134, 135]. Type 2 NASH has been studied in the context of severity, and both adults and children with histology are more likely to have higher stage of fibrosis [136, 137]. It is not clear if this pattern is due to a separate pathophysiological mechanism, though it certainly seems to be a marker of more advanced NASH.

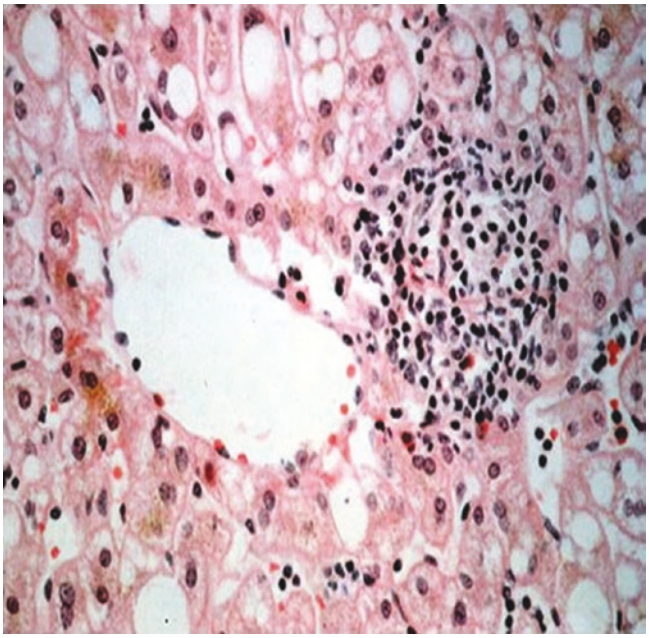

Fig. 69.3 Liver biopsies showing type 1 NASH with pericentral disease. Type 2 NASH is more common in children and type 1 in adults. (Picture kindly provided by Dept. Liver Histopathology, King's College Hospital)

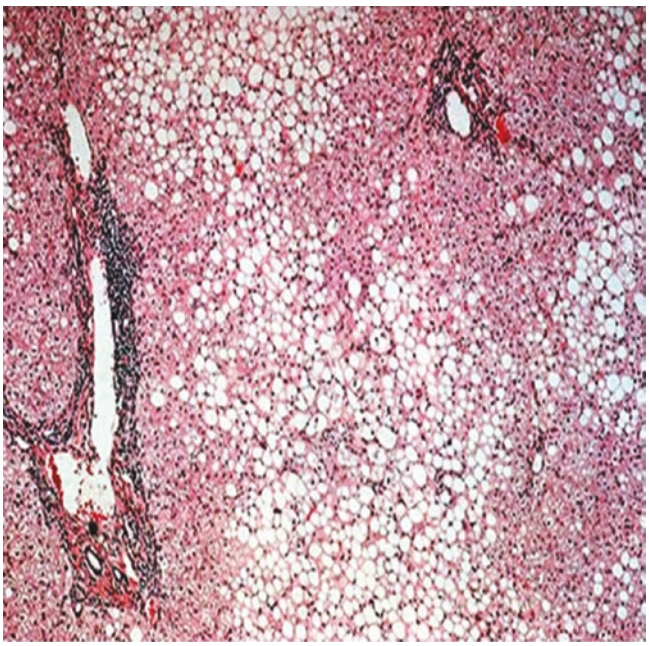

Fig. 69.4 Type 2 NASH with more periportal disease. Type 2 NASH is more common in children and type 1 in adults. (Picture kindly provided by Dept. Liver Histopathology, King's College Hospital)

A possibility which may in part explain the preferential distribution of disease is the concept of zonation. Along the liver lobe, the hepatocytes have different functions depending on their location in the lobule. For example, periportal hepatocytes are functionally specialised in Krebs cycle amongst other tasks, and those in the area of the central vein are rich in cytochrome P450 enzymes. The exposure of the liver to dietary components is more marked in zone 1 than in zone 3 (pericentral) [138].

Africa et al. studied the biopsies of 813 children with NAFLD and found that the presence of periportal (zone 1) steatosis was associated with a younger age and more severe fibrosis. Interestingly, those with zone 3 fibrosis (the more classical adult type 1 pattern) were more likely to have significant inflammation or NASH [137]. The presence of fibrosis in the absence of significant inflammation is now well described leading to the term steatofibrosis as a more accurate reflection of disease stage.

The occurrence of portal inflammation was reviewed as a distinct entity in NASH by the NASH Clinical Research Network (CRN) [136]. A study of biopsies from 728 adults and 205 children found that the presence of portal inflammation in adults was associated with older, female patients with a higher BMI and insulin resistance. There was a clear association with amount and location of steatosis, ballooning and advanced fibrosis. In the paediatric group, portal inflammation was associated with younger age, azonal location of steatosis and more advance fibrosis (bridging). In both groups it was associated with diagnosis of definitive NASH. There was no association with lobular inflammation in either group. It is not clear if this pattern is due to a separate pathophysiological mechanism, though it certainly seems to be a marker of more advanced NASH. The periportal pattern mirrors that of the ductular reaction which has been reported in NAFLD. The possible epithelial-mesenchymal transition of biliary cells in this process may relate to the pattern of fibrosis seen [139].

Though the classic description of fat in NAFLD is macrovesicular, the presence of microvesicular steatosis has been described. In one study approximately 10% biopsies of those in NAFLD revealed microvesicular steatosis [140]. The study found that the presence of microvesicular steatosis was strongly associated with cellular injury and cytoskeletal damage. Microvesicular steatosis has the appearance of distended hepatocytes with foamy cytoplasm; the nucleus is usually central rather than pushed peripherally as in macrovesicular steatosis. Oil red O staining is sometimes needed to identify microvesicular steatosis if it is not visible in haematoxylin and eosin staining. Classically this type of steatosis has been associated with mitochondrial disease, acute fatty liver of pregnancy and some drug effects (e.g. steroids and valproate) which can cause β-oxidation impairment [141]. Taken together, microvesicular steatosis in NAFLD is a likely indicator of mitochondrial damage. It is not yet understood if this is a feature of advanced disease per se or if the pathogenesis of disease in those with microvesicular steatosis is different.

Caldwell et al. reported on the significance of ballooning in NAFLD [142]. These are classically enlarged cells with rarefied cytoplasm. Using ultrastructural analysis this group reported on the multiple small fat lipid droplets seen with degree of ER dilatation and Mallory-Denk bodies and cytoskeletal disarray. Fugii et al. have demonstrated altered expression of FFA-associated protein on the surface of fat droplets which also stain for oxidised phosphatidylcholine (a marker of oxidative damage) [143]. They concluded that oxidative injury to the fat droplet surface may impair its safe disposal and contribute to lipotoxicity.

The Pathology Committee of the NASH CRN proposed a histological scoring system that could be useful in studies of NAFLD [144]. The scoring system includes the evaluation of steatosis (0–3), lobular inflammation (0–2), hepatocellular ballooning (0–2) and fibrosis (0–4). The NAFLD activity score (NAS) is the unweighted sum of steatosis, lobular inflammation and hepatocellular ballooning scores. NAS of 5 or more correlates with the diagnosis of NASH, whilst NAS less than 3 is defined as 'not NASH'. As this system is typically developed for type 1 NASH, the interobserver agreement for type 2 NASH is not as strong (only 18 children were included in the study cohort used for development of the score). The CRN also emphasised that the scoring system was developed as a tool for use in trials and is not a surrogate for a histological diagnosis of NASH [145]. Despite these shortcomings this is the best available tool to standardise the description of the entire spectrum of NAFLD in both adults and children across different centres for research purposes.

It is important, however, to consider the paediatric pattern of disease as a separate entity, particularly when investigating the pathophysiological mechanisms or putative biomarkers of disease severity/progression.

Noninvasive Biomarkers in NAFLD

Though the criterion standard for diagnosis and assessing progression of disease is liver histology, the decision 'if or when' to perform a liver biopsy in children with suspected NAFLD remains controversial. Liver biopsy in children requires admission to hospital and sedation. Risks include bleeding and very rarely death [146]. Repeated biopsy is not a suitable tool for regularly monitoring progression of disease or response to treatment. In addition, biopsy samples only 1/50,000 of the liver, raising the possibility of sampling error [147].

There has been much focus on the development and validation of noninvasive biomarkers of NAFLD in recent years. There is an urgent need for a less invasive method than biopsy of screening the population, stratifying disease severity and following disease progression.

The pathophysiology and evolution of the condition under scrutiny is an important consideration in the development and evaluation of biomarkers. In the case of NAFLD, first is the identification of disease and second the progression. Most longitudinal cohort studies in NAFLD have shown that prognosis is determined by stage and rate of progression of fibrosis rather than the presence of necro-inflammation [52, 148, 149]. Clinical importance lies with being able to differentiate between no/minimal fibrosis (F0/F1), significant fibrosis (F2), severe fibrosis (F3) and cirrhosis (F4).

Serum Biomarkers and NASH

Large adult series have suggested scoring systems using age, BMI, insulin resistance, AST/ALT, platelet count and albumin to differentiate mild from severe disease [150–153]. These simple markers are neither sensitive nor specific enough in isolation [6, 14]. A growing understanding of the pathophysiology of the disease has allowed the investigation of more specific, mechanism-based biomarkers [154–156].

Markers of apoptosis/cell death have been shown to be very useful in differentiating simple steatosis from NASH [112]. CK18-M30 fragments have been shown by a number of studies including paediatric studies to correlate well with severity of NASH [157–160].

Numerous other biomarkers of inflammation, oxidative stress and apoptosis are currently under investigation.

Noninvasive Markers of Fibrosis in NAFLD

Simple tests derived from regression analysis of a large series of patients include the AST to platelet ratio index [161] and the AST to ALT ratio [162], which have been validated in the NAFLD population with AUROC between 0.67 and 0.86 for differentiation of severity of fibrosis [163–165]. Algorithms specifically derived from NAFLD cohorts include the BAAT score (consisting of BMI, ALT, age and triglyceride levels) [166], the BARD score (BMI, AST/ALT ratio, diabetes) [152, 167] and the NAFLD fibrosis score (incorporating age, glucose, AST, ALT, BMI, platelets and albumin) [150, 163, 164, 168, 169]. These markers do not perform particularly well in children however.

In children, Nobili et al. developed and internally validated the paediatric NAFLD fibrosis index (PNFI) in 136 children with NAFLD [170]. Logistic regression analysis of gender, age, BMI, waist circumference, ALT, AST, γGT, albumin, prothrombin time, glucose, insulin, cholesterol and triglycerides gave a predictive model with an AUROC for detection of fibrosis was 0.85. Again this study was limited in view of small numbers in fibrosis groups F2–F4.

The European Liver Fibrosis (ELF) test combining hyaluronic acid, procollagen III N-terminal peptide (P3NP) and TIMP1 was first derived by Rosenberg et al. in a cohort of over 1000 patients with chronic liver disease including NAFLD [171] and has since been validated in other NAFLD cohorts with the addition of several simple markers to improve accuracy [172]. Importantly this test has been shown to correlate well with outcome [173].

The ELF test was evaluated by Nobili et al. in 122 children with NAFLD [174]. Simple markers including age, waist circumference and triglycerides were added to improve diagnostic accuracy. Excellent AUROC for any (0.92), significant (0.98) and advanced (0.99) disease was achieved. In this cohort 37 (30%) had no fibrosis; 58 (48%) scored as F1, 9 (7%) as F2, and 8 (6.5%) as F3–F4. Alkhouri et al. developed this further and validated both the PNFI and ELF in a cohort of 111 children with NAFLD (69% with fibrosis) [175]. The area under the curve for presence of fibrosis was 0.76 for PNFI, 0.92 for ELF and when the two indices were combined: 0.94. The major issue in both studies was the skew towards no or minimal disease, potentially overestimating the accuracy of the test.

Noninvasive Biomarkers and Imaging

Ultrasound, CT and MRI

Ultrasound (US) has a high sensitivity and specificity for diagnosis of steatosis >30%, but is not good at detecting fibrosis. Because of the low cost, the absence of radiation exposure and the wide availability, US is often used in screening for NAFLD. The accumulation of fat causes the liver to appear hyperechoic compared with the kidney. This finding is nonspecific and does not differentiate fat from other substances such as glycogen. When compared with histological findings, the sensitivity of US to detect fat infiltration below 30% of the liver is low [176]. Computed tomography (CT) is rarely used for the assessment of NAFLD in children because of its ionising radiation exposure. Magnetic resonance imaging (MRI) and spectroscopy are the imaging techniques with the greatest accuracy to determine hepatic fat content in studies of both adults and children [129, 177–179]. MRI proton density fat fraction has been validated to liver biopsy with the mean PDFF in those with grade 1 steatosis 9.2%, 15.1% for grade 2 and 26.8% for grade 3 with an acceptable ability to distinguish between stages. Aside from liver fat, however, other features of NASH cannot be assessed. Other methods include MR elastography

which visualises and measures propagating shear waves and has a high sensitivity (>85%) and specificity (>90%) for fibrosis [180]. Cost of this technique may be preclusive however.

For diagnosis of NASH, Iijima et al. have reported on the use of contrast ultrasound with Levovist with an AUC of 1.0 [181]. The decreased accumulation of microbubbles with advancing degree of fibrosis is unique to NAFLD.

There is an emerging literature examining the use of acoustic radiation force-based shear stiffness in NAFLD, an ultrasound-based investigation which uses short bursts of high-intensity acoustic pulses that produce shear waves through the liver tissue, the velocity of which correlates with liver stiffness to correlate well with the stage of fibrosis in the condition [182, 183].

Transient Elastography

Transient elastography (FibroScan) has been shown to be a useful method for detection of liver fibrosis. In NAFLD, a small number of studies have demonstrated the efficacy of TE in distinguishing severity of fibrosis. In a study of 246 adults with NAFLD, TE had an AUROC of 0.84, 0.93 and 0.95 in distinguishing significant fibrosis, severe fibrosis and cirrhosis, respectively [184]. A Japanese study demonstrated similar results [185]. A recent report of 52 children with NAFLD has shown an AUROC of 0.977, 0.992 and 1 for distinguishing any, significant and severe fibrosis [186]. Feasibility and reproducibility of transient elastography is an issue when patients have a BMI > 30 [187]. An XL probe is available for better accuracy in this scenario [188].

Non-hypothesis-Driven Search for Novel Biomarkers Using New Technologies

The use of relatively new techniques such as proteomics [189–192], glycomics [193, 194], lipidomics and metabolomics in the derivation of panels of biomarkers associated with a disease may also give an insight into pathophysiology of the condition.

Natural History and Management

The natural history of paediatric NAFLD has not yet been well described. Case series including one of 20 years describe occasional need for transplantation in young adulthood [195, 196], but the rate of progression is not known [197]. Paired liver biopsies undertaken in 122 children who had enrolled in the placebo arm of two randomised clinical trials in NAFLD were studied. Over time, fibrosis progressed in 23% and improved in 34% [198]. In children who present in the preteenage years with already established with stage 2–3 fibrosis, the rate of progression may be accelerated [199]. The heterogeneity within the population is not yet well understood, but variability in phenotype may be due to underlying genetic susceptibility rather than environmental exposure.

The relative histological severity at presentation in children with this disease and the fact that alcohol is an unlikely confounding factor mean that paediatric NAFLD serves as an excellent disease model in evaluating pathophysiological mechanisms of development and thus targeting intervention in predisposed individuals.

Cirrhosis secondary to NASH has been reported in children as young as 10 years [121, 196]. A recent study by Feldstein et al. describes the long-term outcome of 66 children with NAFLD followed for up to 20 years [200]. Of five children who underwent follow-up biopsy, four showed progression of fibrosis. During the study period, two patients required liver transplantation for decompensated end-stage disease. Both had recurrence of NASH in the allograft and one required retransplantation.

In adult studies, the variables most commonly associated with fibrosis are presence of diabetes, increasing age and high BMI [151]. Similarly in children, severity of obesity and insulin resistance seem to be predictors of advanced fibrosis [22]. The difference between the natural history of type 1 and type 2 NASH has not yet been characterised and is an important subject for future research.

Management of NAFLD encompasses lifestyle modification, medication or both. NAFLD is largely the consequence of imbalanced nutrition and sedentary behaviour on the background of genetic predisposition. Primary prevention is the ideal. In adults, weight reduction of 5–10% body weight often leads to normalisation or improvement of serum transaminases and reduced hepatic steatosis, inflammation and fibrosis [201–203]. In children, weight maintenance as the child crosses the height centiles may achieve the same effect.

In a meta-analysis of adults with NAFLD, weight loss of 5% or more results in improvement in steatosis, whereas ≥7% weight loss resulted in improvement in steatohepatitis, and in those with ≥10% weight loss, all features of NAFLD were reversed or stabilised [204]. In a prospective study again in adults, these outcomes were confirmed [205]. Only 50% of the cohort were able to achieve 7% of weight loss or more though of note in 94% of those who achieved ≥5% weight loss, fibrosis stabilised or reversed.

A small number of trials in children have demonstrated similar outcomes. In a trial of 84 children, weight loss (average 4 kg) over a 12-month period achieved an improvement in ALT and steatosis on ultrasound [135]. Of the cohort, 57 (70%) children completed the 12-month intervention with a mean 8% (SD 4.7%) decrease in weight in the 52 who were overweight or obese. In the remaining five children who

completed the study and had a BMI <85th centile, weight remained unchanged, but ALT levels improved in two and normalised in three patients. Another paediatric study of intensive lifestyle intervention in North America achieved improvement in BMI z-score with a decrease of 0.1 U ($p < 0.05$) baseline to 1 year and decrease in ALT in 69% of the follow-up cohort. There was a 53% dropout rate however [206]. Another study of 53 children comparing lifestyle intervention plus antioxidant or lifestyle intervention plus placebo demonstrated similar improvements in both groups in terms of steatosis, inflammation, ballooning and NAS score [207].

Several case series and uncontrolled trials have demonstrated the effect of weight loss on improvement of transaminases or ultrasound abnormalities [125, 208, 209]. A prospective study carried out in 84 children (3–18.8 years) with NAFLD demonstrated a significant decrease in BMI, levels of fasting glucose, insulin, lipids, transaminases and liver echogenicity on US following a 12-month program of lifestyle advice consisting of diet and physical exercise [135].

Control of both quality and quantity of dietary components may be important. Nutritional data to date has suggested that a high intake of simple carbohydrates such as fructose with a low intake of polyunsaturated fatty acids correlate with pathogenesis and progression of disease [57, 210].

The type of fat consumed is possibly more relevant than quantity with higher saturated fat and lower PUFA intake associated with IR and NAFLD in some studies [56, 210]. Palmitic acid rather than oleic acid results in lower steatosis but in higher cell death and impaired insulin signalling in vitro [61]. Several small studies of PUFAs in adults and one in children have demonstrated improved liver enzymes and histology in the treatment group [211–214].

Insulin resistance is well recognised as an accompanying feature in 70% of those with NAFLD, though not clearly associated with more or less severe disease in terms of inflammation and fibrosis. Insulin sensitisers have been studied frequently in clinical trial but without clear benefit in children. The TONIC trial compared metformin, vitamin E and placebo in 173 children with biopsy-proven NAFLD [215]. There was no statistically significant difference in the outcome measure (improvement in alanine aminotransferase) in those treated with metformin compared to those with placebo. Interestingly a change in HOMA-IR was not seen in those treated with metformin suggesting perhaps that treatment dose may have been insufficient or that selecting out those with insulin resistance may be more appropriate. The likelihood is that as the disease is relatively heterogenous, treatments may need to be individually tailored. Other studies of metformin in children include an open-label pilot study of metformin (500 mg twice daily for 24 weeks) which was conducted in ten nondiabetic children with biopsy-proven NASH and elevated ALT level [216]. Significant improvement was observed in serum ALT and hepatic steatosis as assessed with MR spectroscopy. A subsequent study conducted in children did not show any benefit of metformin compared to lifestyle advice [217]. A third study of metformin in insulin-resistant adolescents resulted in lower severity scores of fatty liver on US and a decrease in prevalence of fatty liver disease in the metformin group [218]. Metformin has the side effect of gastrointestinal intolerance, but weight loss is an advantage of this medication. The thiazolidinediones (TZDs) have the unfortunate side effect of weight gain. There have also been concerns about cardiovascular events and diminished bone mass with their use [219]. There are no available data on the safe use of TZDs in children.

Oxidative stress, most likely mediated by accumulation of fat droplets and the low-grade inflammatory response accompanying visceral adiposity in the setting of genetic predisposition, is known to occur and perpetuate injury in NAFLD. Antioxidants, most commonly vitamin E, have been studied and have been shown in the adult PIVENS trial to reduce steatohepatitis [220] and in TONIC to reduce ballooning. A significant difference in the main outcome measure (ALT) in the paediatric study TONIC was not found however [215].

Current recommendation in adult practice is to use vitamin E in nondiabetic patients with biopsy-proven NASH [221]. The PIVENS study has not yet been validated adequately however, and thus caution is advised generally. There is no consensus on vitamin E use in children with NAFLD.

Ursodeoxycholic acid is another antioxidant which has been used in trial though without consistent effects. Cysteamine bitartrate works by increasing glutathione synthesis. This was used in the CyNCh trial in children with NAFLD in comparison with placebo over 52 weeks. Though there was an improvement in alanine aminotransferase, the primary outcome of histological improvement was not achieved with statistical significance [222].

The gut microbiome and its influence on bile acid metabolism are an exciting area in NAFLD with therapeutic potential. Obesity is associated with a change in the microbiota of the gut and with an increased permeability of the intestinal epithelium [223]. The microbiota influence the development of NAFLD through several different mechanisms amongst which is the production of short-chain fatty acids which stimulate de novo triglyceride synthesis in the liver, modulation of choline metabolism (involved in very low-density lipoprotein synthesis), lipopolysaccharide production and modulation of bile acid metabolism [224].

The change in microbial species leads to a higher concentration of secondary bile acids in the enterohepatic recirculation. In healthy individuals, bile acids play an important role in glucose and lipid metabolism regulating the negative feedback loops. Bile acids act via cellular receptors including

Farnesoid X receptor (FXR) and G protein-coupled bile acid receptor (TGR5) to affect metabolism [224, 225].

Manipulation of gut microbiota with probiotics is a promising way to halt the progression of steatosis to steatohepatitis and fibrosis. Both animal experiments and small human studies have investigated the use of probiotic agents to reverse the progression of steatohepatitis and fibrosis in NAFLD [226, 227]. Two recent double-blind RCTs – one in adults and one in children – were reported [228, 229]. Treatment with probiotics in both studies resulted in improvement in transaminases.

Though probiotics and prebiotics are potentially a way to attempt to re-establish a healthy diversity of flora however, there is no clear guidance on specific formulation nor dose [224]. Weight loss, whether dietary induced or following bariatric surgery, has been found to have a similar effect on the microbiome. Other important ways to disrupt the enterohepatic recirculation of bile thus decreasing the total bile pool is by using FXR agonists [230].

The process of fibrosis is in itself the common end point of several processes: steatosis with oxidative stress, inflammation and apoptosis. Prevention of fibrosis is optimal though antifibrotics are entering the clinical trial arena.

Though there is major interest in the search for a single effective agent for NAFLD, in reality the heterogeneity of the condition means that individualised treatment is required.

Everything considered, the most effective treatment (reversal) of NASH/steatofibrosis is weight loss.

Bariatric Surgery

Bariatric surgery (both Roux-en-Y bypass and gastric band) in management of NAFLD can have beneficial effects in adults showing improvement in AST, ALT, NAFLD fibrosis score and NAFLD activity score post-operatively [231, 232]. In children and young people, Manco et al. described a comparison of sleeve gastrectomy to intragastric balloon and lifestyle advice only and found reversal of NASH at 1-year post surgery in 100% of those who underwent sleeve gastrectomy [214].

New Agents

There are dozens of novel and repurposed therapies currently under investigation for a therapeutic benefit in NAFLD. Amongst these are a peroxisome proliferator-activated receptor alpha and gamma agonist elafibranor that acts as an insulin sensitiser, obeticholic acid a synthetic chenodeoxycholic acid analogue which is an agonist for FXR, losartan (which can decrease PAI1 expression) and antifibrogenic agents.

Why does lifestyle intervention not work for all? Adherence may be the principal challenge, and given that children and young people are almost entirely dependent on family being involved in their goal, this increases the complexity of problem.

Social deprivation, a lack of education about the importance of a healthy lifestyle and cost of fresh, unprocessed foods are all important considerations and not all which can be successfully managed in the context of a clinical lifestyle intervention programme.

An important yet underexplored area in ability to undertake and maintain lifestyle change in this population is the importance of mental health. The prevalence of depression and anxiety in children and young people and adults with obesity is high [233, 234]. Depression is also overrepresented in adults with NAFLD with 53% exhibiting subclinical depression and 14% clinical depression, 45% with subclinical anxiety with 25% clinical anxiety in a large cohort study [235]. A small number of studies have shown that quality of life (QoL) in both adults and children with NAFLD is consistently inferior to normal controls. In a survey of 239 children apart of the NASH CRN, children with NAFLD had worse total physical and psychological quality of life scores as determined by the PedsQL questionnaire. Fatigue, trouble sleeping and sadness accounted for almost half the variance in QoL scores [236]. Though obstructive sleep apnoea (OSA) may contribute to daytime somnolence in obesity, non-OSA sleep problems and the pathogenesis of this fatigue have not yet been systematically studied in NAFLD.

Response to lifestyle intervention in NAFLD is affected adversely in the presence of depression [237]. Though some studies in adults have used a psychological approach to lifestyle change in obesity-related disorders, no trials in children are reported [238–240].

There is a distinct link between poor mental health/depression, inflammation and the coexistence of complications of obesity [241–243]. The effect of visceral, inflammatory obesity leading to exaggerated or prolonged inflammatory responses compounded by sickness behaviours, depressive symptoms and poor lifestyle choices may lead to further inflammation and consequent end-organ damage [241]. Effective treatment options targeting this vicious cycle may halt both the amplified inflammation and depressive symptoms.

Future Areas for Research

The pathophysiology of NAFLD in both adults and children is still incompletely understood. The reason for different patterns of disease in children, particularly with reference to the occurrence of periportal inflammation and fibrosis rather

than the typical type 1 pattern of pericentral disease, remains elusive. Determining susceptibility to the disease using genetic analysis for SNPs may become standard practice, particularly in screening the high number of overweight and obese paediatric patients. Further investigation into specific dietary patterns in children may also yield valuable information in this multifactorial disease.

The determination of the most effective management of the condition – both in terms of achieving lifestyle change and pharmacological treatments – will be a major focus going forwards. Further areas of interest include the role of intestinal microbiota and the possible use of probiotics in the condition and dietary manipulation with PUFA.

Finally the long-term outcome of children with NAFLD remains unknown, and well-designed, long-term prospective studies using networks such as the NASH Clinical Research Network in the US are vital to achieving this.

The recognition of NAFLD in children is important in order to prevent progressive disease through young adulthood. Though closely associated with overweight and obesity, genetic and epigenetic clearly lead to susceptibility to the disease in the setting of an unhealthy lifestyle. At presentation 15% of children will have bridging fibrosis and thus a relatively severe degree of liver injury. Liver injury is reversible however, and lifestyle change which addresses a mismatch in energy balance is fundamental but achievable in less than 50% of those undergoing an intensive programme with dietary advice and support. The reasons for this are as yet unclear but in part may be mediated by the high prevalence of depression and anxiety in those with this disease. There is a growing industry in treatments which target one of more pathophysiological process involved. Stratifying patients with NAFLD according to their susceptibilities and recognising comorbid mental health problems will facilitate individualised therapy and hopefully more successful outcomes.

In conclusion, NAFLD in children is a very real, very prevalent condition and, should current trends continue, is likely to become the most common indication for liver transplantation in coming decades. It is important that the condition is recognised in children as there is the potential to reverse the condition and to avoid the morbidity and mortality associated in later years.

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Vascular Disorders of the Liver

70

Ruth De Bruyne and Pauline De Bruyne

Portal Vein Anomalies

The portal vein (PV), originating at the union of the splenic vein (SV) and superior mesenteric vein (SMV), drains the blood from the gastrointestinal tract, the spleen, pancreas, and biliary apparatus [1, 2]. Between the fourth and tenth week of embryonic life, the PV develops from an anastomotic network formed by the vitelline veins around the duodenum [1, 3, 4]. Congenital anomalies of the PV are rare and can often be explained by persistence of portions of the vitelline veins [5].

Congenital Anomalies of the Portal Vein

The most common congenital anomaly of the PV is a *preduodenal portal vein (PDPV)* in which the PV passes anteriorly to the duodenum rather than posteriorly [5–9]. The embryogenesis of this anomaly, described by Gray and Skandalakis [10], consists of the persistence of a preduodenal vitelline communicating vein [11, 12]. Although PDPV can occur as an isolated defect, it is typically associated with other congenital anomalies, including heterotaxia or polysplenia syndrome [13–18], situs inversus [14, 18, 19], cardiac defects [14, 16, 20], malrotation [7, 14, 16–19, 21–24], biliary or duodenal atresia [14, 18, 25], and annular pancreas [14, 17]. Clinically, PDPV can cause a duodenal obstruction by itself or in combination with the coexisting anomalies. Approximately 50% of patients remain asymptomatic, with PDPV being a radiologic or peroperative incidental finding [6, 7, 14, 17, 18]. PDPV is of surgical importance because it may cause difficulties in procedures of the gallbladder, biliary ducts, and duodenum [8, 13, 14, 17, 20, 23, 25].

Congenital PV atresia or hypoplasia can involve the whole length of the PV or can be localized just proximal to its division into its two main branches in the porta hepatis. The foetal umbilical vein and ductus venosus which empty into the left PV undergo a spontaneous obliterative process at birth. If this obliterative process is excessive, the involvement of the PV may lead to PV atresia or hypoplasia [26]. This phenomenon often occurs in patients with biliary atresia [27, 28]. Children with small or hypoplastic PV represent a challenge for liver transplantation with higher risk of complications (such as thrombosis, stenosis, and liver graft ischaemia) [28].

The PV is the most common site of visceral venous aneurysms [29–31]. Nevertheless, *PV aneurysms* (PVAs) are rare, representing fewer than 3% of all venous aneurysms [29, 32–34]. Extrahepatic and intrahepatic locations have been described. The major location of PVA is the main extrahepatic PV at the confluence of the SV and SMV [31–34]. Some PVAs are thought to be congenital: it has been proposed that incomplete regression of the distal right primitive vitelline vein leads to a vascular diverticulum that ultimately develops into an aneurysm [29, 32, 33]. Portal hypertension, related to chronic liver disease, is the most common acquired aetiology of PVA [29–31, 33]. Other acquired causes are inflammatory processes (such as pancreatitis, trauma, invasive malignancy) and previous surgical intervention [30, 35, 36]. The most common clinical presentation is nonspecific abdominal pain, followed by incidental finding and gastrointestinal bleeding [31, 33, 37]. Complications of PVA include PV thrombosis and rupture, biliary tract compression, duodenal compression, and inferior caval vein obstruction [29, 31, 33, 38]. Management ranges from watchful waiting (with serial ultrasound imaging studies) to intervention. Surgical intervention is considered for symptomatic patients, patients

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with complications, or patients with enlarging aneurysms [29, 31, 33, 39].

Anomalous pulmonary venous return is a congenital cardiac malformation in which the pulmonary veins fail to connect with the left atrium during cardiac development [40, 41]. In the infradiaphragmatic type of this anomaly, the pulmonary veins drain through a large vessel into the portal venous system or the inferior vena cava [2, 4, 42]. This anomaly is frequently associated with complex cardiac defects [40, 43]. Clinical symptoms of this infradiaphragmatic total anomalous pulmonary venous drainage commonly develop early (within 24–36 h of life) and include respiratory distress with cyanosis, tachypnoea, and tachycardia [41, 44]. No corrective treatment with catheterization exists for infradiaphragmatic anomalous pulmonary venous return. The goal of surgery is to redirect pulmonary venous flow entirely to the left atrium [41, 42].

Abnormal connections between the portal and hepatic veins (portosystemic shunts) and between the hepatic artery with the PV (arterioportal shunts) are discussed in the 'Hepatic Vascular Shunts' section.

Extrahepatic Portal Vein Obstruction

Definition and Aetiology

Extrahepatic PV obstruction (EHPVO) is a major cause of portal hypertension in children and adolescents [45–51]. In EHPVO, the portal inflow is hindered by congenital or acquired obstruction of the PV, commonly by portal vein thrombosis [52–55]. This results in cavernomatous replacement of the PV [32, 53], which consists of formation of venous channels that act as portoportal collateral vessels within and around an occluded PV. The cavernoma consists of dilated biliary (cystic and pericholecystic veins) and gastric branches (left and right gastric veins) of the PV, and the partially recanalized thrombus. These collaterals are usually insufficient to bypass the whole splenomesenteric inflow resulting in signs of prehepatic portal hypertension [32].

Portal vein thrombosis (PVT) is usually associated with the presence of a hypercoagulable state, vascular injury, or stasis [5]. Prothrombotic conditions should be excluded in children presenting with an EHPVO. Genetic abnormalities affecting the physiologic anticoagulant system, such as hereditary deficiency of protein C, protein S, and antithrombin as well as factor V Leiden, methylenetetrahydrofolate reductase C677T, and prothrombin G20210A mutations, are thought to be risk factors of venous thrombosis in adults and should be investigated in children and adolescents with PVT [46, 50, 52, 56, 57].

Direct damage to the PV in about 25% of cases can be linked to a history of umbilical vein catheterization during the neonatal period. Umbilical catheters may cause thrombosis by damage to vessel walls, disruption of blood flow, and damage to endothelial cells by the infusion of substances such as total parenteral nutrition [51, 52, 57]. Portal hypertension appears to be rather uncommon following neonatal PVT. This may be due to the predominant left PV involvement [51, 52].

Clinical Presentation

The presentation of PV thrombosis may be acute or chronic. *Acute PV thrombosis* should be suspected when patients present with symptoms such as abdominal pain, ascites, or fever in the absence of portal cavernoma and portosystemic collaterals. However, patients can also be asymptomatic [56]. Most children with *chronic EHPVO* eventually develop hypersplenism that requires medical assessment. Markedly, one-third to one-half of the children present with acute upper gastrointestinal bleeding with no prior history of gastrointestinal disorders or symptoms of hypersplenism. Morbidity of chronic EHVPO is mainly related to hypersplenism, portal biliopathy, limitations of quality of life (e.g. limited ability to participate in sports because of extreme thrombocytopenia and/or splenomegaly), growth retardation, and neurocognitive impairment (also described as minimal hepatic encephalopathy) [55, 56, 58–63].

Diagnosis

EHPVO is diagnosed by Doppler ultrasound (US), computed tomography (CT), or magnetic resonance (MR) angiography [35, 49, 56, 64] which demonstrate PV obstruction, presence of intraluminal material, or PV cavernoma (Fig. 70.1). CT or MRI imaging enables a good evaluation of the patency of the other abdominal veins facilitating planning of potential future intervention [35, 55]. When patency of the intrahepatic PV remains uncertain, transjugular retrograde or percutaneous transhepatic portal venography should be undertaken. Further diagnostic workup consists of liver biopsy when liver disease is suspected, full hypercoagulability panel, and echocardiography to look for congenital heart disease and for signs of hepatopulmonary syndrome or portopulmonary hypertension [56].

Management

Management of acute PV thrombosis can involve treatment of the causative factor and anticoagulation. Anticoagulation therapy should be considered for patients with a prothrombotic condition. Management of patients with chronic

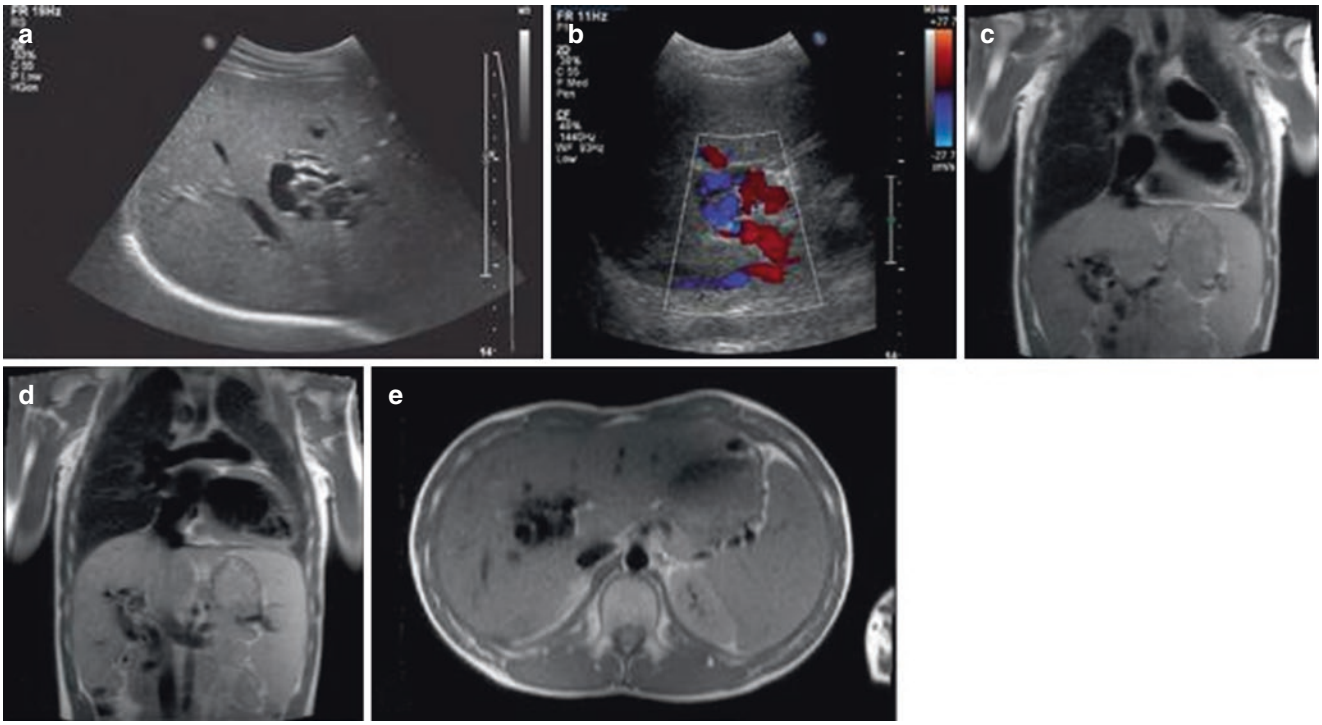

Fig. 70.1 Ultrasonography (**a**) and colour Doppler (**b**) showing cavernomatous transformation of the portal vein in a 14-year-old boy with portal vein thrombosis. Liver MRI illustrating coronal (**c**, **d**) and axial (**e**) T_2-weighted HASTE images in the same patient. *HASTE* half-Fourier single-shot turbo spin echo (Courtesy of Prof. dr. D. Voet, Department of Ultrasonography, and Prof. dr. N. Herregods, Department of Radiology, Ghent University Hospital, Belgium)

EHPVO focusses on management of oesophageal varices, hypersplenism, and portal biliopathy [55, 60]. Monitoring of growth retardation and neurocognitive impairment is also important in their follow-up [55, 60]. Patients with severe morbidity may need (shunt) surgery [35, 60, 65]. The current shunt technique of choice for children presenting with EHPVO is – if technically and anatomically feasible – mesocaval shunt (Rex shunt) placement, connecting the superior mesenteric vein to the left portal vein in the region of the Rex recess [2, 60].

For the detailed management of portal hypertension caused by EHPVO, refer to Chap. 71 'Portal Hypertension in Children'.

Hepatic Artery Anomalies

Ischaemic Cholangiopathy

Ischaemic cholangiopathy has been defined as focal or extensive damage to bile ducts due to impaired blood supply [66]. Unlike the hepatic parenchyma which has a blood supply from both the hepatic artery and PV, the biliary system only depends on the arterial blood supply [5, 66]. Ischaemic bile duct injury may occur when small hepatic arteries or the peribiliary plexus is injured (by iatrogenic factors or by systemic conditions such as vasculitis), or when all arterial blood supply is interrupted as in the case of hepatic artery thrombosis after liver transplantation [67]. In the acute stage, patients may present with pain, fever, and jaundice. In the further course, focal or diffuse bile duct stenosis can occur with variable presentation: from no symptoms to (fluctuating) jaundice, itch, fatigue, or bacterial cholangitis and eventual portal hypertension [66]. Treatment of ischaemic cholangiopathy must be individualized in relation to symptoms, acute or chronic presentation, and the location of biliary injury [67].

Pseudoaneurysm of the Hepatic Artery

Pseudoaneurysm of an artery is a type of haematoma that develops between the two outer layers of the artery, the tunica media and tunica adventitia; this is in contrast with true aneurysms involving all three layers of the wall of an artery [68, 69]. Hepatic artery pseudoaneurysms (HAPs) are commonly iatrogenic [70] or a result of abdominal trauma [64]. HAPs can be found incidentally. On the other hand, rupture of the pseudoaneurysm can be the first clinical manifestation with abdominal pain, gastrointestinal haemorrhage,

or haemobilia [69, 71]. Although HAPs can resolve spontaneously by thrombosis, the reported risk of rupture ranges from 14% to 80% [72, 73]. Hence, once HAP is diagnosed, the lesion should be treated regardless of symptoms. Treatment consists of embolization or stent placement, or surgical approach [69, 72].

Abnormalities of the Sinusoidal Blood Flow

Pericellular Fibrosis

Pericellular fibrosis is commonly seen in alcoholic liver disease, chronic passive congestion, nonalcoholic fatty liver disease, Gaucher's disease, congenital syphilis, and vitamin A toxicity [5]. The hepatic sinusoids comprise one of the largest-calibre vascular beds in the body. Impairment of blood flow through this vascular bed results in a major loss of physiologic function, with profound influence on homoeostasis for the entire human organism [74]. Endothelial dysfunction occurs early in chronic liver disease even before fibrosis and inflammation take place, and persists in advanced cirrhosis which is the most common cause of sinusoidal blood flow obstruction [75].

Physical Occlusion of the Sinusoids

In sickle cell disease, sinusoids can become packed with sickled red cells and erythrophagocytes leading to parenchymal necrosis [76]. In disseminated intravascular coagulation and eclampsia, fibrin deposits may occlude the sinusoids. When these lesions are severe, widespread infarction might occur. Furthermore, the sinusoids might become infiltrated by mast cells in mastocytosis, Gaucher's cells, metastatic tumour cells, and leukaemia or lymphoma cells [5].

Peliosis Hepatis

Peliosis hepatis is a rare tumour-like condition in which the sinusoidal dilatation is primary [77]. The liver parenchyma contains multiple blood-filled cystic spaces, either nonlined or lined with sinusoidal endothelial cells [78]. The disease is more common in adults, and the exact pathogenesis of peliosis hepatis is unknown [74, 79]. In adults, peliosis hepatis can be associated with the prolonged use of several drugs such as anabolic steroids, azathioprine, and oral contraceptives, [80], or presents in the context of chronic underlying disorders such as malnutrition [81], leukaemia [82], tuberculosis [83], vasculitis [84], cystic fibrosis, malignancy [85], organ transplantation [86], and human immunodeficiency virus (HIV) infection [87]. Peliotic lesions found in acquired immunodeficiency syndrome (AIDS) and other immunosuppressed patients are caused by bacterial organisms (*Bartonella* species) [82, 88]. In 20–50% of patients, peliosis hepatis is idiopathic [89]. In children, peliosis hepatis also occurs most frequently in association with chronic underlying conditions such as cystic fibrosis [90], malnutrition [91], Fanconi anaemia [92], adrenal tumours [93], Marfan syndrome [94], congenital cardiopathy [95], myotubular myopathy [96, 97], and renal transplantation [80]. In paediatric cases without underlying systemic disorder, an association with *Escherichia coli* infection suggesting a direct role of *E. coli* toxins in causing endothelial damage [98–100] has been reported. The definitive diagnosis of peliosis hepatis is based on histological findings but should be suspected when ultrasonography reveals hypoechogenic areas involving the whole liver in association with intraperitoneal fluid and normal Doppler signals [99]. Percutaneous liver biopsy carries a high risk of life-threatening haemorrhage. Therefore, a surgical or transjugular approach appears more appropriate [101].

Hepatic Vein Anomalies

Budd–Chiari Syndrome (BCS)

BCS is defined as hepatic venous outflow obstruction at any level from the small hepatic veins to the junction of the inferior vena cava and the right atrium, regardless of the cause of obstruction. Outflow obstruction caused by hepatic venoocclusive disease and cardiac disorders is excluded from this definition. BCS can be classified as primary due to an endoluminal venous lesion (thrombosis or webs) or secondary due to intraluminal invasion by a parasite or malignant tumour or extraluminal compression by an abscess, cyst, or solid tumour [102]. BCS is very rare in children accounting for approximately 0.1% of paediatric liver disease in Western countries in contrast to 7.4% on the Indian subcontinent [103, 104]. In primary BCS, an underlying prothrombotic disorder or established risk factor for venous thrombosis is often present. In adults, myeloproliferative diseases account for half of the cases of BCS [105, 106]. Children usually have a chronic presentation, and prothrombotic conditions are present in two-thirds of these cases. Table 70.1 gives an overview of predisposing conditions for BCS. The role of hyperhomocysteinaemia and primary protein C, protein S, or antithrombin deficiency is unclear because liver disease or recent thrombus formation and consumption can obscure recognition of these disorders [106]. Hepatic venous outflow tract obstruction results in increased sinusoidal pressure, sinusoidal congestion, hepatomegaly, hepatic pain, portal hypertension, and ascites. Elevated sinusoidal pressure leads to perisinusoidal necrosis of hepatocytes in the centrilobular

region and eventually to irreversible liver damage, cirrhosis, or liver failure [107].

A diagnosis of BCS should be considered in any patient who presents with acute or chronic liver disease as the clinical manifestations can be extremely diverse. The majority of patients present with the typical triad described by George Budd in 1845: right upper quadrant pain, hepatomegaly, and ascites. Oedema of the lower extremities is also a common finding [102]. Asymptomatic BCS accounts for 15–20% of cases [108]. Compared to adults, adolescents more often present with hepatomegaly alone [109]. Jaundice, gastrointestinal bleeding, and hepatic encephalopathy are less common [110]. The diagnosis of BCS is established upon demonstration of obstruction of the hepatic venous outflow tract. US combined with Doppler imaging has a diagnostic sensitivity of more than 75% and should be the first line of investigation [111, 112]. Hepatic veins devoid of flow signal, collateral hepatic venous circulation, a spider web appearance usually located in the vicinity of the hepatic vein ostia and stagnant, reversed, or turbulent flow can all be indicative of BCS [113, 114]. When adequate ultrasonography is technically difficult or when diagnostic features cannot be demonstrated, CT or MRI should be performed. The latter is preferred in children due to absence of radiation exposure. Only in a minority of cases, retrograde cannulation of the hepatic veins for X-ray venography will be ultimately necessary for diagnosis. This technique allows the assessment of the extent of venous outflow obstruction and allows for pressure measurements (Fig. 70.2). Concurrent liver biopsy can contribute in confirming the diagnosis and ruling out other causes such as veno-occlusive disease and cirrhosis of other aetiologies [115]. Once the diagnosis of BCS is established, the patient should be investigated for underlying prothrombotic conditions, and a haematologic workup for myeloproliferative disorder should be performed. The rarity of BCS in children often means that the disease is diagnosed in its later stages by which time irreversible pathology may be present [116].

The management of BCS requires early referral and a multidisciplinary approach consisting of anticoagulation, angioplasty, and thrombolytic therapy, with or without stenting, transjugular intrahepatic portosystemic stent shunting (TIPSS), surgically fashioned portosystemic shunts, and orthotopic liver transplantation [117]. Thrombolytic therapy might be successful in dissolving fresh thrombi and can be

Table 70.1 Predisposing conditions for Budd–Chiari syndrome

Inherited conditions
Factor V Leiden mutation
G20210A prothrombin gene mutations
Hyperhomocysteinaemia
Primary protein C or protein S deficiency
Antithrombin deficiency
Acquired conditions
Myeloproliferative disorders (V617 JAK2 positive)
Antiphospholipid syndrome
Behcet's disease
Paroxysmal nocturnal haemoglobinuria
Environmental factors
Oral contraceptive use
Toxins like heavy metals, aflatoxins, etc.

JAK2 Janus kinase 2

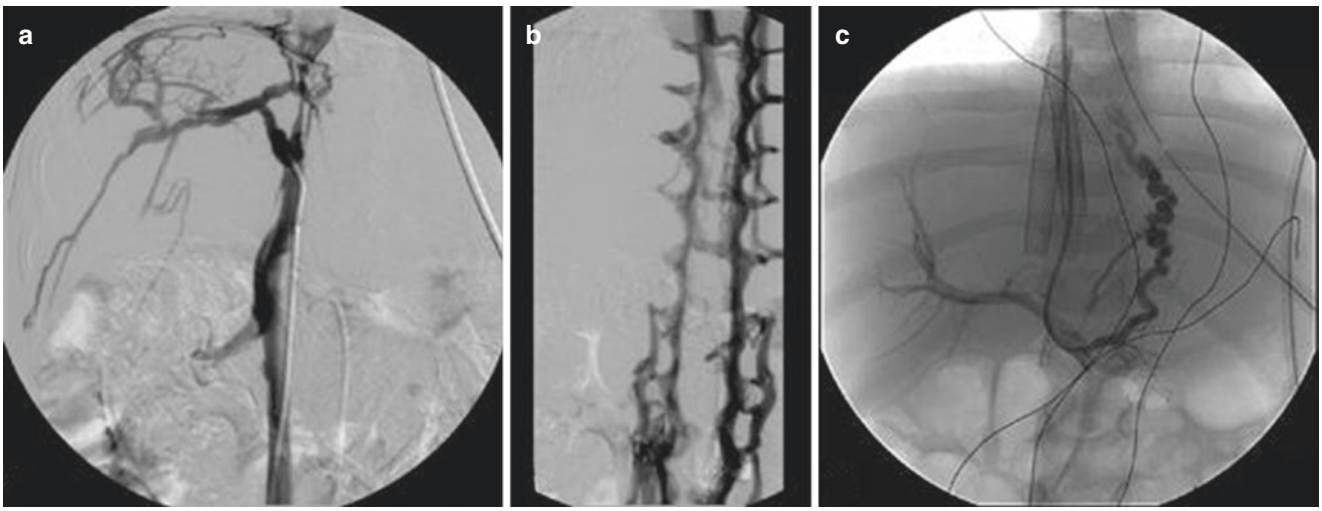

Fig. 70.2 Congenital stenosis of the inferior caval vein with Budd–Chiari syndrome in a 4-year-old child. (**a**) Cranial cavography with catheter tip at caudal site of inferior caval vein stenosis with visualization of collateral circulation at the level of the liver capsule. (**b**) Caudal cavography illustrating collateral circulation via the lumbar veins caused by the more cranially positioned stenosis. (**c**) Placement of stent in inferior caval vein and TIPSS with direct venography of oesophageal varices. *TIPSS* transjugular intrahepatic portosystemic stent shunting. (Courtesy of Prof. dr. P. Van Langenhove and Prof. dr. L. Defreyne, Department of Interventional Radiology, Ghent University Hospital, Belgium)

combined by transjugular balloon angioplasty. Maintenance of flow can be ensured by long-term anticoagulation, and if present, the underlying haematologic disorder should be managed medically. TIPSS has been successfully used in children with favourable long-term results [118–120]. Percutaneous stent placement has also played a role in the management of paediatric BCS [121, 122] (Fig. 70.2). Redkar and colleagues published their experience in a case series of 25 paediatric BCS patients. 21 underwent balloon angioplasty of which 17 had good medium- to long-term results, while only 1 out of 4 patients who underwent portocaval shunts survived [123]. The authors encourage early aggressive use of interventional radiological procedures, but further multicentre prospective studies are required to establish diagnostic and therapeutic management protocols for paediatric BCS [124]. In acute liver failure or a late presentation with established hepatic cirrhosis and complications of portal hypertension, liver transplantation is the only option that can offer an excellent chance of long-term survival [103, 125–127].

Veno-Occlusive Disease (VOD)

Hepatic VOD, also known as sinusoidal obstruction syndrome (SOS), is a severe and potentially fatal liver disease originally described in Jamaican drinkers of pyrrolizidine alkaloid-containing bush tea. It is a potentially life-threatening complication following haematopoietic stem cell transplantation (HSCT) or conventional chemotherapy and radiotherapy not associated with HSCT [128].

Many of the cytotoxic agents used in HSCT are metabolized in the liver, including cyclophosphamide and busulfan. It is thought that depletion of glutathione in zone 3 hepatocytes and sinusoidal cells plays an important role in the initiation of the damage [129]. Toxicity from chemotherapy or radiotherapy can lead to sinusoidal endothelial cell activation due to the production of pro-inflammatory cytokines by injured tissues, infection, medications, or the process of bone-marrow engraftment. This can result in damage to the sinusoidal endothelial barrier with obstruction of the sinusoidal flow and ultimately hepatocellular necrosis, fibrosis, vascular occlusion with liver failure, hepatorenal syndrome, multi-organ failure, and death [130, 131].

The prevalence of VOD in children, adolescents, and young adults varies from 20% up to 60%, compared to approximately 10% in adults [128, 132–136]. Children undergoing HSCT for specific diagnoses such as osteopetrosis, high-risk neuroblastoma, thalassaemia, and congenital macrophage activation syndrome are specifically at risk for VOD with a prevalence up to 60% [132–136]. Other risk factors are young age (<1 year), treatment regimens containing busulfan or combination treatments with monoclonal antibodies (e.g. inotuzumab ozogamicin, gemtuzumab ozogamicin), second allogeneic HSCT, etc. [137–143].

The clinical course is typically characterized by rapid weight gain, abdominal pain, hepatomegaly, and ascites. Jaundice, which is an important diagnostic item in the Baltimore and Seattle criteria [144, 145] used in adults, may be absent in 30% of paediatric patients or may appear late in the course of VOD [132]. Early diagnosis and intervention are necessary to prevent multi-organ failure with its very high mortality rate (>80%) [146].

Since the disorder differs significantly between adults and children in terms of incidence, genetic predisposition, clinical presentation, and treatment, the European Society for Blood and Marrow Transplantation (EBMT) proposed new diagnostic guidelines in 2017 [147]. The EBMT paediatric criteria have no limitation for time of onset of VOD and require two or more of the following: unexplained consumptive and transfusion-refractory thrombocytopenia; otherwise unexplained weight gain on three consecutive days despite the use of diuretics, or a weight gain of more than 5% above baseline value within 72 h; bilirubin increasing from baseline on three consecutive days, or bilirubin 2 mg/dL or more within 72 h; hepatomegaly (best if supported by imaging) above baseline value; and ascites (best if supported by imaging) above baseline [147].

In addition to the EBMT diagnostic criteria, an international expert position statement, consensus recommendations were provided for the international implementation of guidelines for the diagnosis, severity grading, and treatment of VOD among children, adolescents, and young adults [128].

In view of its favourable safety profile and promising results in both prospective randomized and retrospective studies, the use of ursodeoxycholic acid in the prophylaxis of VOD is recommended [148, 149]. It is recommended that defibrotide (25 mg/kg per day) is started once diagnostic criteria for VOD are met [128]. The therapeutic results are better than in adults [147]. Guidelines on the use of defibrotide prophylaxis in VOD are awaited, but defibrotide prophylaxis can be considered for high-risk paediatric patients. Supportive treatment of VOD focusses on maintaining intravascular volume and renal perfusion without increasing extravascular fluid accumulation. Avoidance of exposure to hepatotoxic drugs, fluid and sodium restriction, and diuretics are important in the care of a patient with VOD.

Congestive Cardiac Failure

Due to congestive cardiac failure, blood will pile up in the hepatoportal venous system, leading to sinusoidal dilatation, splanchnic congestion, and portal hypertension. The distension of hepatic sinusoids results in parenchymal atrophy,

nodular regenerative hyperplasia (NRH) [150], and stimulation of the fibrotic response which can lead to cirrhosis, liver failure, and increased risk of hepatocellular carcinoma [151].

Hepatic Vascular Shunts

Arteriovenous Malformations

Arterioportal shunts may be congenital (associated with hereditary haemorrhagic telangiectasia, Ehlers–Danlos syndrome, or biliary atresia) or acquired (after blunt or penetrating trauma, after percutaneous liver biopsy, or with cirrhosis) and consist of a communication between the hepatic artery and the portal venous system [2, 5, 32, 41, 152]. Most congenital arterioportal fistulas are symptomatic by the age of 2 years [153, 154]. Hepatofugal flow develops in the arterialized PV that can lead to presinusoidal portal hypertension, hypersplenism, varices, ascites, and hypertensive enteropathy [41, 152–154]. Treatment of arterioportal shunts is either surgical or minimally invasive through interventional embolization techniques [152–155].

Hepatic arteriosystemic shunts are the rarest form of intrahepatic shunts. These shunts connect the hepatic artery (or other systemic arteries) and the hepatic veins. These shunts may be congenital or associated with hereditary haemorrhagic telangiectasia, hepatocellular carcinoma, or large haemangiomas [32]. Hepatic arteriosystemic shunts are usually localized in one lobe of the liver [41]. Arteriovenous fistulas can not only present clinically in neonates with congestive heart failure, anaemia, hepatomegaly, and portal hypertension but also later in childhood in hereditary haemorrhagic telangiectasia with congestive heart failure, hepatic ischaemia, and portal hypertension [41, 156]. Currently, supportive measures, interventional closure (in localized lesions), and surgery (including liver transplantation) are the main treatment methods for hepatic arteriosystemic shunts [41, 156–158].

Portosystemic Shunts

Portosystemic shunts are characterized by abnormal vascular communications between the portal venous system and systemic venous system [2]. The complicated development of the inferior caval vein and the close relationship of its development with that of the vitelline veins may explain the occurrence of congenital portosystemic shunts (CPSS). Incidence of CPSS is estimated to be 1:30,000 [159–161]. CPSS are associated with several congenital anomalies and syndromes. Various forms of congenital heart disease, vascular anomalies, and heterotaxy are clearly linked to CPSS. Associations with Turner syndrome, Down syndrome, and Noonan syndrome and associations with genitourinary, abdominal, and musculoskeletal anomalies have been described [162–164]. Acquired portosystemic shunts occur as a consequence of portal hypertension [165, 166].

Both extrahepatic and intrahepatic portosystemic shunts have been described [41, 167]. Congenital *extrahepatic portosystemic venous shunts* are also known as Abernethy malformations described in 1793 [1, 168–171]. Morgan and Superina classified extrahepatic portosystemic shunts into two types [168, 171]. In type I, there is a complete diversion of the portal blood flow in the caval vein, with congenital absence of the PV. This is further divided into type Ia (the SV and SMV draining separately into a systemic vein) and type Ib (common trunk of PV draining into a systemic vein) [1, 5, 35]. In type II, the PV is intact, but some of the portal flow is diverted into the caval vein through a side-to-side extrahepatic communication [35, 41]. Congenital *intrahepatic portosystemic shunts* are abnormal intrahepatic anastomoses between branches of the PV and the hepatic veins, which develop due to persistence of communication between vitelline veins and sinus venosus [5, 35, 172] (Fig. 70.3). Intrahepatic shunts can occur in one or in both lobes and consist of one or multiple portosystemic connections [163].

Portosystemic shunts allow mesenteric blood to reach the systemic circulation bypassing the liver. This can lead to serious complications such as hepatic encephalopathy, hepatopulmonary syndrome, and pulmonary hypertension, and development of liver tumours. Therefore, screening for asymptomatic complications and close surveillance in CPSS patients are needed [164]. Portosystemic venous shunting causes increased levels of galactose, ammonia, and other nitrogenous substances in the plasma, some of which can negatively affect the brain, with development of encephalopathy [2, 32, 164, 173]. The development of hepatopulmonary syndrome and pulmonary hypertension may be explained by exposure of the pulmonary vascular bed to intestinal vasoactive mediators that have bypassed the liver [163, 164, 174]. Hepatic complications of CPSS include the development of benign and malignant liver tumours, most commonly in patients with extrahepatic CPSS. Reported liver lesions include focal nodular hyperplasia, regenerative nodular hyperplasia, hepatoblastoma, hepatic adenomas, haemangiomas, and hepatocellular carcinoma [162–164, 175–179].

Ultrasonography with Doppler study is usually the first-line exam that shows abnormal communications between the portal and the systemic veins and the consequences on liver morphology. When CPSS is suspected, CT or MRI imaging of the abdomen is indicated to assess the presence of a shunt, the anatomy of the shunt, the patency of the portal vein, and the presence of liver masses [162, 175]. Because of challenging and widely variable portal vein identification in imaging studies (CT or MRI), angiography with occlusion

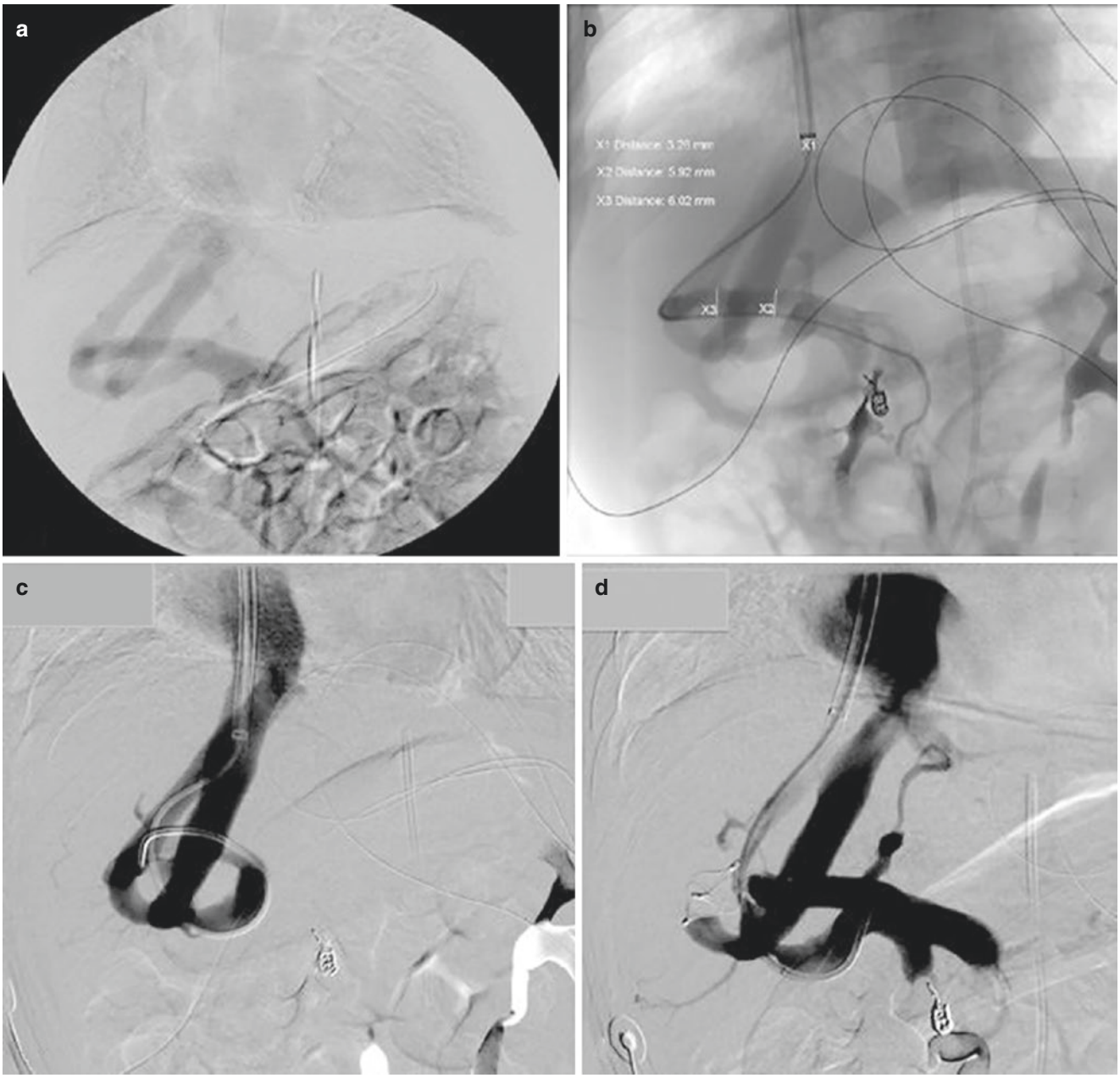

Fig. 70.3 A 5-month-old child with multiple intrahepatic portosystemic shunts. (**a**) Indirect mesentericography with visualization of multiple intrahepatic portosystemic shunts. (**b**) Direct portography in the same patient with guiding into the right hepatic vein. The child previously presented with acute haematemesis caused by a bleed from a pseudoaneurysm of the gastroduodenal artery which was coiled in a previous procedure. (**c**) Illustration of interconnections in the complex portosystemic shunt. (**d**) Direct portography after occlusion of one connection of the portosystemic shunt with vascular plug. (Courtesy of Prof. dr. P. Van Langenhove and Prof. dr. L. Defreyne, Department of Interventional Radiology, Ghent University Hospital, Belgium)

test is essential in most patients and is stated to be the gold standard diagnostic [165, 180–182].

Patients with a CPSS should be followed in a specialized centre. Importantly, therapeutic approach should be individualized: shunt type, patient age, and the presence and severity of symptoms determine treatment strategy [182, 183]. Spontaneous closure of CPSS is mainly described in patients with intrahepatic shunts and occurs mostly within the first year of life. Therapeutic procedures for intrahepatic CPSS should therefore be postponed in asymptomatic infants younger than 1 year [35, 163, 165, 180]. There still remains debate regarding therapeutic strategies for asymptomatic cases of CPSS beyond the age of 1 year. In view of the risk of future complications, several authors state that

preventive treatment may be indicated for asymptomatic CPSS [161, 164, 165, 183]. The choice of a surgical or an interventional radiology shunt closure technique for intrahepatic and extrahepatic CPSS depends on local expertise, the anatomy of the shunt, and the condition of the patient [163, 180, 182]. Liver resection and transplantation is a therapeutic option, especially in patients with specific extrahepatic or large multifocal intrahepatic shunts that are not eligible for embolization or in patients with liver tumours [162, 163, 182, 184].

Hereditary Haemorrhagic Telangiectasia

Hereditary haemorrhagic telangiectasia (HHT) or Rendu–Osler–Weber disease is an autosomal dominant vascular disorder with variable penetrance with an estimated prevalence of 1: 5000–8000 [185]. Clinical manifestations are the result of telangiectasias and arteriovenous malformations involving the mucocutaneous tissues, lung, brain, and liver [2, 186]. The pathophysiological mechanism depends on anomalous angiogenesis with aberrant vascular responses to injury-induced and other angiogenic stimuli [186]. Hepatic vascular malformations have been reported in 47% of paediatric patients with HHT ranging from small telangiectases to large arteriovenous malformations, with various stages of severity [187].

Parenchymal Response to Vascular Injury

Nodular Regenerative Hyperplasia (NRH)

NRH is a major cause of non-cirrhotic portal hypertension in the Western world. It is a benign condition characterized by diffuse transformation of the liver parenchyma into small regenerative nodules distributed evenly throughout the liver with minimal or no fibrosis in the perisinusoidal or periportal areas [150]. The nodules vary in size, from a few millimetres to several centimetres. It is hypothesized that NRH results from disturbances in the intrahepatic venous, arterial, or sinusoidal microcirculation resulting in ischaemic atrophy and compensatory regeneration [188–190]. NRH occurs predominantly in older patients between 25 and 60 years and is rather uncommon in children [191–198]. In a large series of 716 paediatric liver tumours, NRH was demonstrated in 4.5% of the cases [199]. NRH can be idiopathic or can be associated with various multisystem diseases affecting the hepatic blood flow including solid-organ transplantation; haematopoietic stem cell transplantation; vasculitic conditions; autoimmune, inflammatory, and neoplastic diseases; or HIV [74]. Cytotoxic and immunosuppressive drugs such as azathioprine, 6-mercaptopurine, and 6-thioguanine may induce NRH [200–202]. NRH presenting with progressive portal hypertension was described in six children treated with 6-thioguanine as maintenance therapy for childhood acute lymphoblastic leukaemia [203]. The disease is often discovered incidentally during abdominal imaging performed for various reasons [190]. In advanced stages portal hypertension is the most often associated finding. Imaging findings are relatively poor in sensitivity and specificity for NRH. Regenerative nodules are often not visible on ultrasound [189] or can appear as multiple, tiny, well-circumscribed, homogeneous, and hypo-/isoechoic lesions. A diffusely heterogeneous hepatic parenchyma or distortion of the hepatic architecture may be the only imaging abnormality [190]. On CT, regenerative nodules remain isodense or hypodense in both arterial and portal venous phases, distinguishing NRH from FNH and adenomas [191]. The significance of MRI in the diagnosis of NRH is still controversial. Lesions appear slightly hyperintense on T_1-weighted images and iso- or hypointense on T_2-weighted images [204]. The nodules may present gadolinium enhancement preferentially in the portal venous phase, similar to normal liver parenchyma. This is an important feature for distinguishing NRH nodules from other focal liver lesions which may present similar imaging aspects (like FNH, hepatic adenoma, or metastatic disease) and demonstrate arterial phase enhancement, while NRH does not [190]. The gold standard for diagnosis is histopathology demonstrating regenerative nodules consisting of hypertrophied hepatocytes centrally surrounded by atrophic hepatocytes peripherally (Fig. 70.4. There is no or minimal perisinusoidal or portal fibrosis on reticulin staining, and compression of the central veins by the regenerating nodules may be seen [205, 206]. The management of NRH mainly relates to the prevention and treatment of complications of portal hypertension.

Focal Nodular Hyperplasia (FNH)

FNH is a well-circumscribed, non-neoplastic lesion characterized by benign-appearing hepatocytes with vascular anomalies and ductal proliferation of unknown aetiology [190, 207]. Vascular malformations, vascular injuries, and increased flow after paediatric oncologic therapy have all been suggested as underlying mechanisms [190]. The diagnosis of FNH is rarely made in the paediatric population (only 15% of all FNH cases) [208]. It most often affects females between the age of 30 and 50 years [209, 210]. Most cases are found incidentally on abdominal imaging. Large subcapsular lesions might lead to vague abdominal pain. Complications such as rupture and intratumoural haemorrhage are extremely rare. Most lesions remain stable with a likelihood to regress with age, and there is no malignant potential [208]. On imaging, FNH should be differentiated

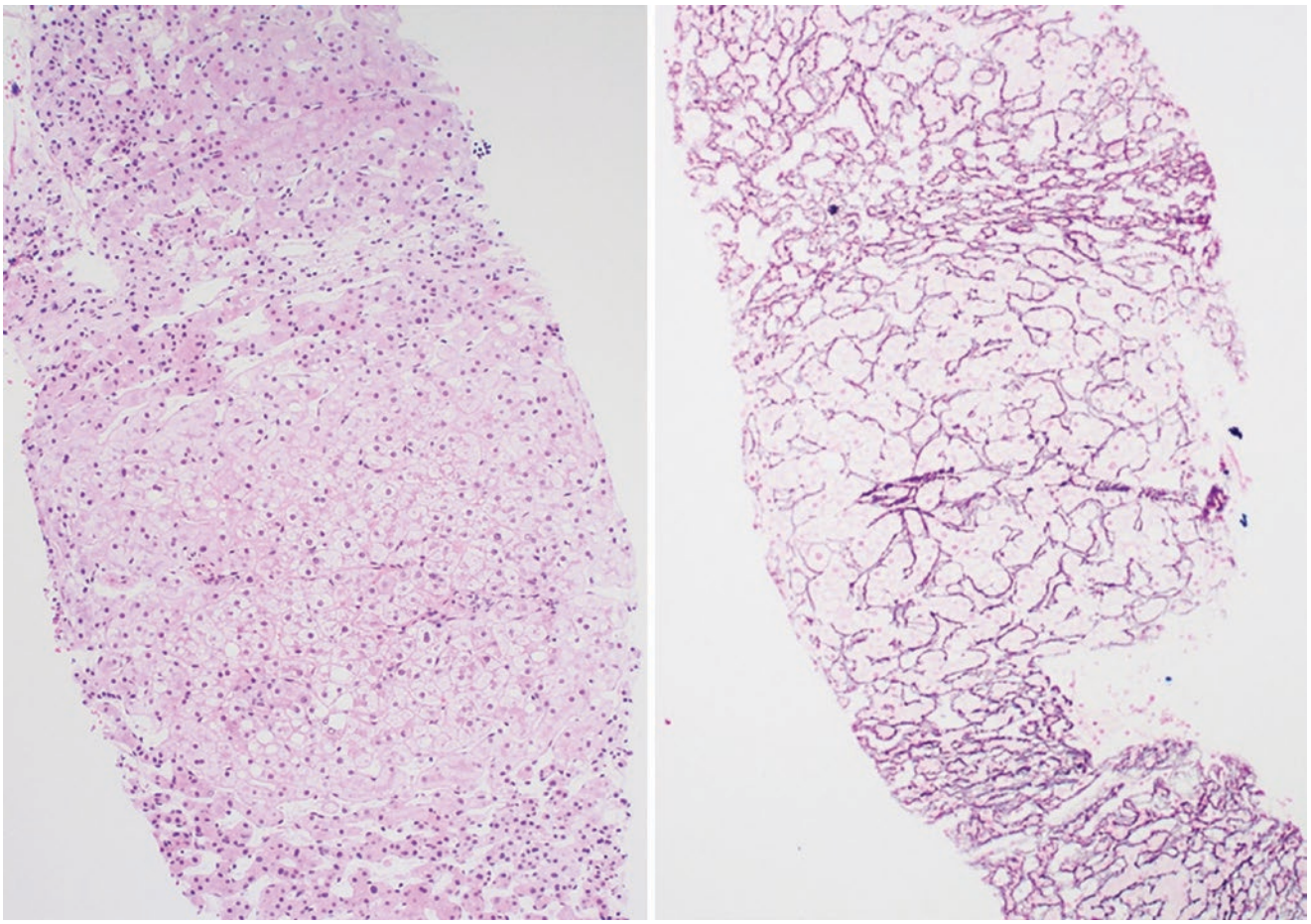

Fig. 70.4 Nodular regenerative hyperplasia (NRH): Liver biopsy of a 16-year-old girl with cystic fibrosis and portal hypertension. H&E staining shows a nodule composed of regenerative hepatocytes surrounded by atrophic hepatocytes forming thinned plates. A reticulin stain clearly outlines the nodule as well as the compressed and atrophic hepatocyte cords surrounding the nodule. There was no fibrosis. (×100) (Courtesy of Prof. dr. A. Hoorens, Department of Pathology, Ghent University Hospital, Belgium)

from liver cell adenoma [211] or hepatocellular carcinoma [212]. The typical ultrasound finding in FNH is a well-demarcated homogeneous hypo- or isoechoic lesion with a central scar which can be seen in less than 20% of cases. On colour Doppler, there is a central arterial structure with a spoke-wheel pattern of radiating smaller aberrant vessels [213]. CT shows typically a well-circumscribed iso- or hypodense lesion with rapid homogeneous intense enhancement in the arterial phase and gradual enhancement of the central scar in the portal venous phase [214]. On MRI, a homogeneous lesion is seen that is isointense or slightly hypointense on T_1- and isointense or slightly hyperintense on T_2-weighted images. During the arterial phase, the typical FNH lesion becomes homogeneously hyperintense apart from the central scar which often exhibits avid enhancement in the delayed phase [215] (Fig. 70.5). In the presence of typical radiological findings, there is usually no indication for liver biopsy. When performed, histopathology (Fig. 70.6) shows a nonencapsulated nodule with a central stellate fibrous region containing large vessels from which there are radiating septa. The parenchyma between the septa exhibits essentially normal hepatocytes but with a thickened plate architecture characteristic of regeneration [209]. Patients with asymptomatic FNH are treated conservatively without need for further imaging if there is a typical radiological appearance of FNH. In case of symptoms, which are usually seen in large subcapsular lesions, or in case of biochemical abnormalities or atypical radiological features which do not allow to rule out malignancy, surgical resection can be indicated [208]. Growth alone should not be considered as an indication for surgery [216].

Benign and Malignant Vascular Tumours

Vascular tumours of the liver comprise a substantial portion (13%) of all hepatic neoplasms in children [217]. Most of these lesions are benign. *Infantile hepatic haemangioma* (IHH) is the most common benign tumour of the liver in infancy [218]. It is recommended to avoid the terminology

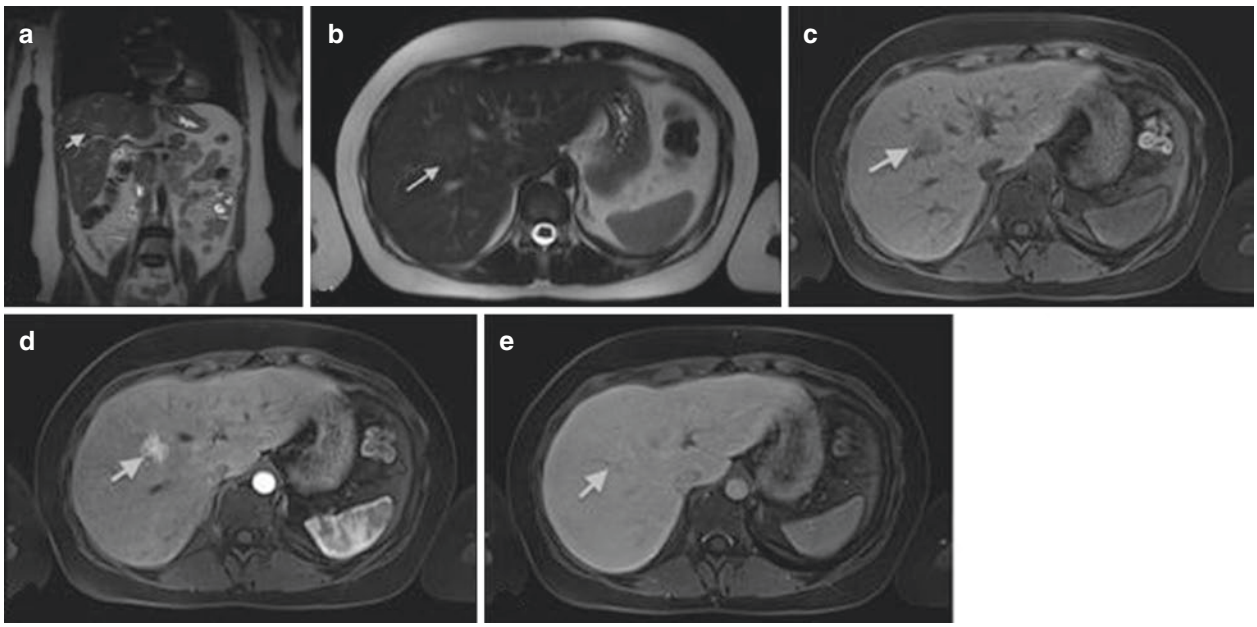

Fig. 70.5 MRI showing focal nodular hyperplasia (FNH) in a 16-year-old girl. This lesion is discretely hyperintense on T2-weighted images (**a**, **b**), hypointense on T1-weighted images (**c**), with intense early arterial phase enhancement (**d**) and isointense aspect compared to normal liver parenchyma in the portovenous phase (**e**). (Courtesy of Prof. dr. N. Herregods, Department of Radiology, Ghent University Hospital, Belgium)

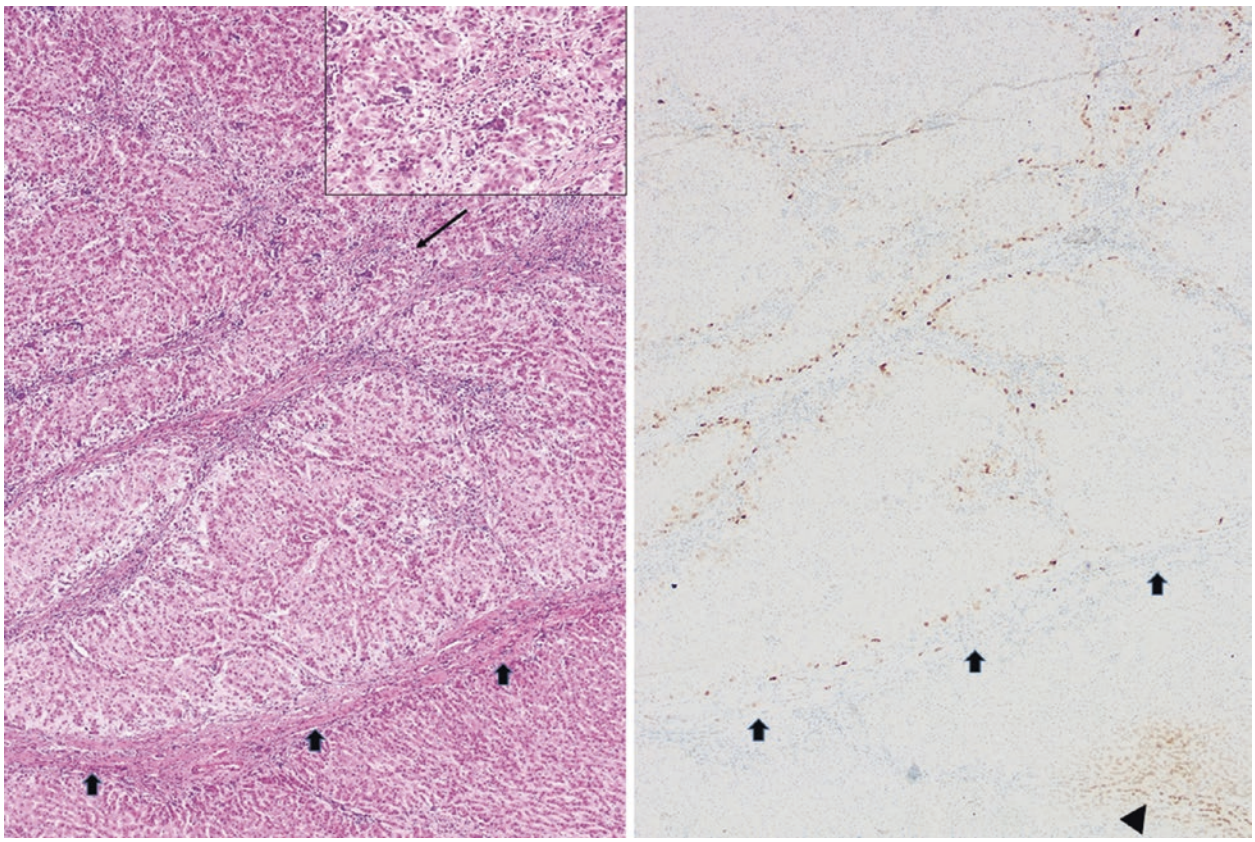

Fig. 70.6 Focal nodular hyperplasia-like lesion (FNH-like lesion) in an 18-year-old boy more than 15 years after bone marrow transplantation. H&E staining shows a nodule with fibrous septa with ductular reaction. Short arrows indicate the limits between FNH-like nodule and non-tumoral liver. (×40) Inset: higher magnification of the area indicated with a long arrow showing the ductular reaction. (×100) These FNH-like lesions may not show the characteristic map-like pattern of glutamine synthetase (GS) staining seen in true FNH, as in shown in this patent. Arrowhead indicates normal GS staining around a central vein in the non-tumoral liver. (×40) (Courtesy of Prof. dr. A. Hoorens, Department of Pathology, Ghent University Hospital, Belgium)

infantile haemangioendothelioma, which is a histological diagnosis (and therefore requires histological evaluation) and might be confused with kaposiform and epithelioid haemangioendothelioma. The majority of IHH presents within the first 6 months after birth and involute until 3–9 years of age [158, 219–223]. The incidence of IHH is unknown as a substantial part remains asymptomatic and often undiagnosed [224]. IHH can manifest as hepatomegaly, palpable abdominal mass, or distension. Life-threatening congestive heart failure can occur due to high cardiac output as well as haemolytic anaemia, thrombocytopenia, and consumption coagulopathy. Infantile HH express type 3 iodothyronine deiodinase that converts thyroid hormone to its inactive form. This can result in acquired hypothyroidism which is often seen in larger multifocal and diffuse IHH and resolves with tumour involution [225–230]. Other symptoms are jaundice, liver failure, intra-abdominal bleeding due to tumour rupture, respiratory distress, and pulmonary hypertension. Approximately half of the cases also have cutaneous haemangiomas. Based on data from the Liver Haemangioma Registry, a division into three principal categories has been proposed: focal, multifocal, and diffuse lesions [224, 227, 231]. Focal lesions could be considered as the hepatic variant of the cutaneous rapidly involuting congenital haemangioma which typically evolves during foetal life and is fully grown at birth. These lesions do not expand postnatally and are less commonly associated with accompanying cutaneous infantile haemangioma [220, 232, 233]. Because they develop antenatally, focal IHH can be diagnosed prenatally [234–236]. Most focal IHH are discovered as an abdominal mass in an otherwise healthy child and involute rapidly by the age of 12–14 months [227]. Ultrasonography reveals a well-circumscribed mass with large feeding and draining vessels. On CT or gadolinium MRI, a well-defined, solitary, spherical tumour with centripetal enhancement and central sparing because of thrombosis, necrosis, or intralesional haemorrhage can be seen [158, 224, 231] (Fig. 70.7). Multifocal and diffuse HH are considered as true IHH [227]. They are associated with cutaneous infantile haemangiomas

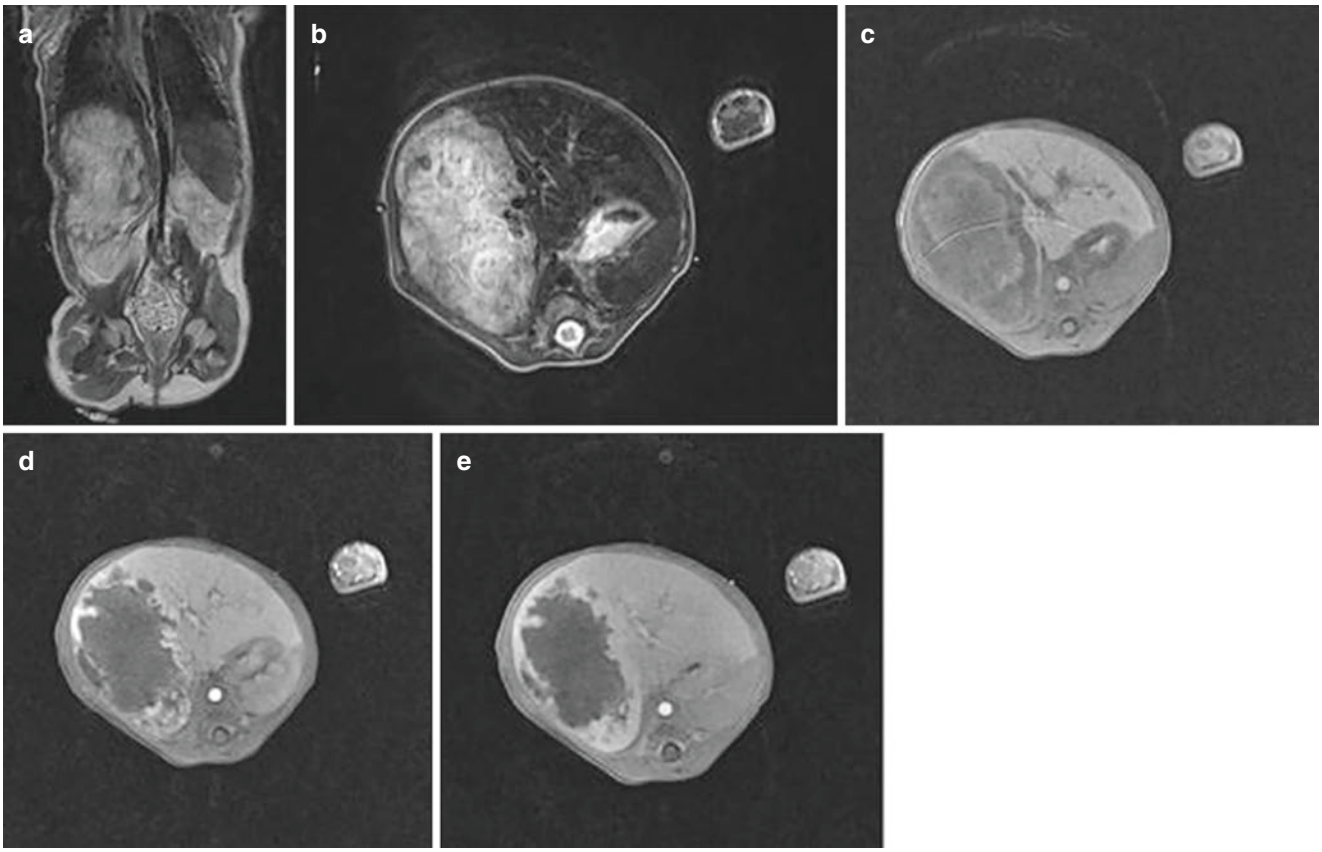

Fig. 70.7 MRI findings in a 2-week-old infant with infantile hepatic haemangioma. Large, relatively well-demarcated exophytic mass in the *right* lobe of the liver (81 × 49 × 87 mm). There is heterogeneous hyperintensity on T_2-weighted images with prominent flow voids (**a**, **b**). On non-contrast-enhanced T_1-weighted images, the spontaneous hyperintense zones in the lesion are compatible with haemorrhagic components (**c**). After intravenous contrast administration, there is peripheral nodular enhancement of the lesion in the arterial phase (**d**) with progressive, centripetal filling in the portovenous phase (**e**). (Courtesy of Prof. dr. N. Herregods, Department of Radiology, Ghent University Hospital, Belgium)

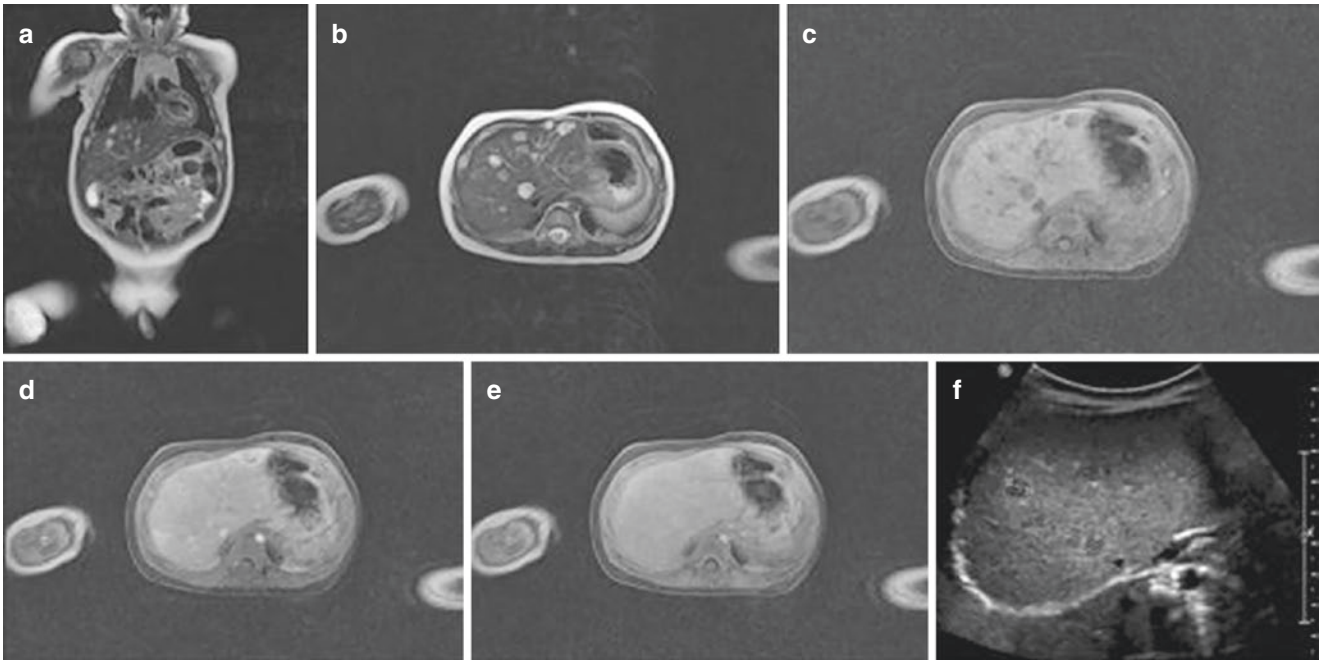

Fig. 70.8 MRI of multifocal infantile hepatic haemangioma in a 3-month-old boy. Multiple well-defined T_2-hyperintense (**a**, **b**), T_1-hypointense (**c**) nodular lesions spread throughout the liver. After intravenous contrast administration, there is nodular enhancement in the arterial phase (**d**) with nearly isointense aspect of the lesions in the portovenous phase compared to unaffected liver (**e**). US findings in the same child (**f**). (Courtesy of Prof. dr. N. Herregods, Department of Radiology, Ghent University Hospital, Belgium)

in the majority of cases and characterized by the immunoexpression of glucose transporter (GLUT)-1 in liver tissue [227]. These lesions appear within the first weeks of life and are therefore not antenatally detected. The typical course in these lesions is one of rapid postnatal growth (0–12 months) followed by slow involution (1–5 years) [227]. Multiple well-defined spherical lesions are observed on CT, MRI, or ultrasound with intervening areas of normal hepatic parenchyma in multifocal IHH, whereas the lesions in diffuse IHH nearly totally replace the liver [227] (Fig. 70.8). The diagnosis of IHH is based on medical history, physical examination, and imaging. Pathological examination is rarely necessary but should not be delayed in the face of diagnostic uncertainty or when children present with vascular tumours after infancy [158, 227]. Two histological subtypes have been described (Fig. 70.9). Type 1 IHH are composed of multiple small vascular channels with an immature endothelial cell lining and a fibrous stromal separation containing bile ductules between the channels. Type 2 IHH have a more disorganized appearing endothelial cell lining and no stromal bile ductules [217, 223, 237]. The treatment of IHH depends on clinical presentation, tumour subtype, size, anatomy, and the severity of complications [222]. Asymptomatic IHH should be observed by serial US follow-up. In case of symptoms, medical treatment, interventional radiology, or surgery is indicated. Corticosteroids were previously considered as the first-line medical treatment. However since 2014, oral propranolol, a nonselective β-blocker, was approved by the European Medicines Agency and became the medical treatment of choice for IHH requiring systemic therapy [238–240]. The dosage ranges from 2 to 3 mg/kg/day in 2 or 3 doses for 6 months. In case of haemodynamic instability and when there is no response to pharmacological treatment, arterial embolization, hepatic artery ligation, or surgical resection should be considered. Hepatic transplantation may be indicated in extremis when other treatment options are impossible or fail [227]. *Congenital hepatic haemangiomas* (CHH) are less common than IHH, proliferate in utero, and are fully formed at birth. They can be either rapidly involuting (by 2 years of age), partially involuting, or non-involuting. Histologically, they are GLUT-1 negative and do not express lymphatic markers [241]. As stated above, some authors pose that focal IHH might represent rapidly involuting CHH as they also stain negative for GLUT-1. *Paediatric angiosarcoma* is a very rare but highly malignant tumour. It usually presents with a rapidly growing hepatic mass. The precise diagnosis may be difficult, even on a biopsy specimen [242]. Open biopsy of the tumour is therefore advisable. Most angiosarcomas develop after the first year of life. Radical resection and even liver transplantation should be attempted but are often impossible due to the extent of the tumour or metastatic disease. Chemotherapy (ifosfamide, doxorubicin, and vincristine or gemcitabine and docetaxel) usually follows resection. Several gene-targeting therapies such as tyrosine kinase inhibitors (sorafenib, sunitinib, pazopanib) and anti-vascular endothelial growth factor monoclonal antibodies

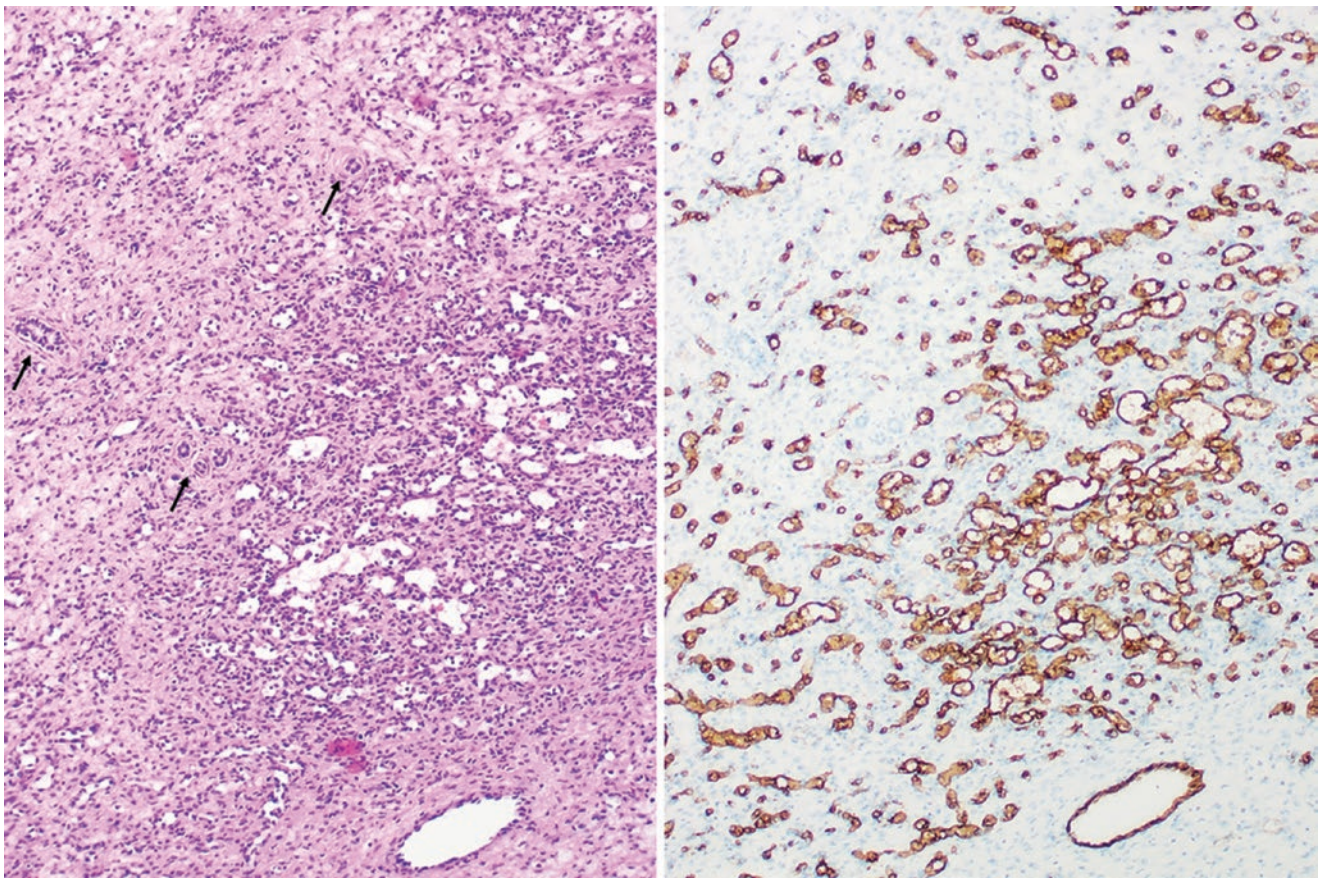

Fig. 70.9 Infantile haemangioma in a 2-month-old boy. H&E staining and immunohistochemistry for the endothelial marker CD34 shows a tumour composed of variable sized vascular structures lined by a single layer of flat to somewhat plump endothelial cells without significant atypia. Arrows indicate entrapped ductular structures. (×100) (Courtesy of Prof. dr. A. Hoorens, Department of Pathology, Ghent University Hospital, Belgium)

(bevacizumab) have been used with some success [243, 244]. The prognosis remains very poor. *Hepatic haemangioendothelioma* is a rare neoplasm of endothelial origin having a clinical behaviour intermediate between haemangioma and angiosarcoma [245, 246]. Patients generally present from the second decade, and the tumour commonly affects the liver, lung, skin, or bone, but also other presentations are reported [247].

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Portal Hypertension in Children

71

Angelo Di Giorgio and Lorenzo D'Antiga

Introduction

Portal hypertension (PH) is the commonest complication of chronic liver disease in children as in adults [1]. Patients with PH are at risk of complications including splenomegaly, bleeding from intestinal varices, and ascites. Children with PH and their parents may be frightened by the most severe complication of PH, gastrointestinal hemorrhage (GH), often referred to as a terrifying experience and giving the impression of impending death; gastrointestinal bleeding in children has high morbidity, nevertheless, only few pediatric patients die from variceal bleeding, especially if they have a non-cirrhotic cause of PH [2]. Unfortunately, there are very few robust data published in the past few decades in children with PH [3]. Conversely, in adults, many treatments have been challenged, and a plethora of studies have been carried out and summarized periodically in the Baveno Consensus Conference [4]. A panel of experts sporadically provides a pediatric opinion on the Baveno Conference trying to translate the experience gained from treating adults into children [3–5].

Therefore, most children are treated simply extrapolating the data from adults and applying the same protocols adapted according to body size [6, 7]. Nevertheless, there are several important differences between PH in children and adults. One is represented by the early onset and rapid progression of pediatric liver diseases causing cirrhotic PH, together with a relatively larger availability of split organ donations allowing us to solve most of the severe cases by organ replacement. Thus, the length of the follow-up of children with severe cirrhotic PH is short. Second, a large proportion of children with PH have presinusoidal disease, having different implications as far as management and outcome are concerned [8]. These points explain why most of the children with large varices and bleeding that we manage in the long term usually have non-cirrhotic PH, clearly a different scenario from that seen in adult practice.

Anatomy of the Portal Venous System

The liver receives blood from two main vessels: the proper hepatic artery and the portal vein. The former is a branch of the common hepatic artery, which arises from celiac trunk, and supplies oxygenated blood accounting for 25% of the blood entering the liver. The latter, which drains deoxygenated blood accounting for 75% of the liver blood flow, is the largest vessel of the portal venous system. In adults, the total hepatic blood flow ranges between 800 and 1200 mL/min, which is equivalent to approximately 100 mL/min per 100 g of wet liver. Although the liver mass constitutes only 2.5% of the total body weight, this organ receives nearly 25% of the cardiac output.

This huge portal venous flow is driven through the liver across a minute pressure gradient. The pressure gradient between the portal inflow and the hepatic venous outflow is usually no more than 5 mmHg. The resistance to blood flow through the portal vein is so low because of the unique hepatic vasculature, with conducting blood vessels terminating in each of the microvascular units of the acinus and flowing past only approximately 20 hepatocytes before exiting into the wide hepatic venules. Thus, at least 50% of the entire blood content of the liver can be expelled without significant vascular resistance [9].

The portal venous system extends from the intestinal capillaries to the hepatic sinusoids. It carries the blood from the abdominal gastrointestinal (GI) tract, pancreas, gallbladder, and spleen back to the heart flowing through the liver (Fig. 71.1).

In this system, the central vessel is the portal vein, which is formed by the union of the splenic vein (SV) and the superior mesenteric vein (SMV), but receiving blood also from

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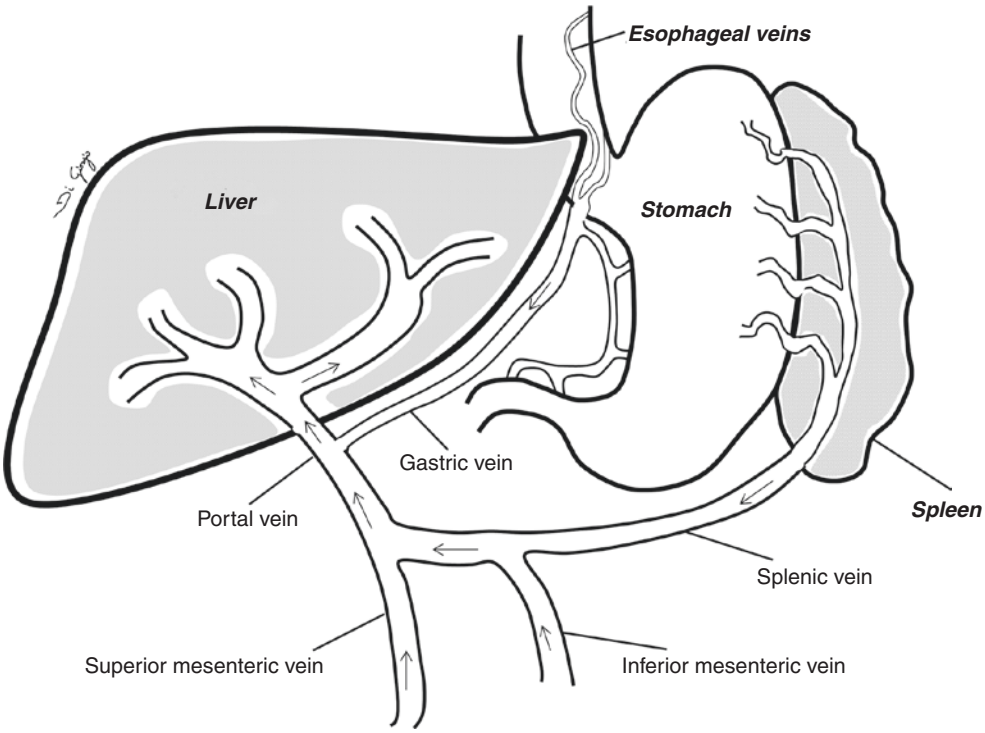

Fig. 71.1 Anatomy of the portal system

the inferior mesenteric vein (IMV), the gastric, and the cystic veins. The SMV is formed by tributaries from the small intestine, colon, and head of the pancreas, and irregularly from the stomach via the right gastroepiploic vein. The SVs originate at the splenic hilum and join near the tail of the pancreas with the short gastric vessels to form the main SV. The IMV carries blood from the left part of the colon and rectum and reaches the SV in its medial third. Anatomical variations include the IMV draining into the confluence of the SMV and the SV, and the IMV draining in the SMV (Fig. 71.1).

Immediately before reaching the liver, the portal vein divides into right and left main branches, and then ramifies further, forming smaller venous branches, and ultimately the portal venules. Each portal venule runs alongside a hepatic arteriole and the two vessels form the vascular components of the portal triad. These vessels ultimately merge into the hepatic sinusoids to supply blood to the liver. Three hepatic veins (right, middle, and left) drain the blood from the liver into the inferior vena cava (IVC) [10].

Pathophysiology of Portal Hypertension

The portal venous pressure is directly proportional to the portal blood flow and the hepatic resistance, according to Ohm's law ($\Delta P = Q \times R$, where ΔP is the variation of pressure along the vessel, Q is the blood flow, and R is the resistance to flow). Since portal vascular resistance is inversely proportional to the fourth power of the radius (Poiseuille's equation), a small decrease in the vessel diameter produces a large increase in the portal vascular resistance and, in turn, in portal blood pressure. In the healthy liver, the intrahepatic resistance changes according to the variation of portal blood flow to keep portal pressure within normal limits. In fact, under physiological conditions, a rise in portal pressure is counteracted by sinusoidal dilatation, even in the presence of increased blood flow as can happen after meal ingestion [10, 11]. Most of the following statements made on PH come from experiments on animal models, such as the rat with a ligated portal vein or bile duct or with carbon tetrachloride-induced cirrhosis, and then confirmed in clinical studies carried out mainly in adults [11–13].

Increased Vascular Resistance

The portal venous system has a baseline portal pressure of 7–10 mmHg, and the hepatic venous pressure gradient (HVPG) ranges from 1 to 4 mmHg. PH is defined as a portal pressure greater than 10 mmHg or a gradient greater than 4 mmHg. In adults, a pressure gradient above 10 mmHg has been associated with esophageal varix (EV) formation, and with ascites and variceal bleeding if above 12 mmHg [14, 15].

The main pathogenetic factor in the development of PH is an increased vascular resistance (Fig. 71.2). Depending

on the site in which it occurs, PH can be classified as extrahepatic (prehepatic and posthepatic) and intrahepatic. The latter may be further subdivided into three forms including presinusoidal (portal venules), sinusoidal (sinusoids), and postsinusoidal (terminal hepatic venules; central veins; Table 71.1).

In patients affected by chronic liver disease, though, increased vascular resistance is located at various intrahepatic levels.

The pathogenetic mechanism explaining the increased resistance in extrahepatic PH, where the blood flow is blocked by a mechanical obstruction, is quite obvious. Conversely, the pathogenesis is more complicated in intrahepatic PH, in which many factors, both mechanical and dynamic, may occur simultaneously [15].

Increased Portal Blood Flow

The second factor contributing to the development of PH is an increase in blood flow, established through splanchnic arteriolar vasodilatation caused by an excessive release of endogenous vasodilators including nitric oxide (NO), glucagon, and endothelin (activated by the vasoactive intestinal peptide), as well as by the activation of the sympathetic and the renin–angiotensin systems. These changes cause sodium and water retention, hypervolemia, renal hypoperfusion, and increase in cardiac output and splanchnic blood inflow, resulting in a hyperdynamic vascular status which characterizes the advanced stages of PH (Fig. 71.2).

Extrahepatic Causes of PH

Prehepatic causes of increased resistance to flow include SV thrombosis, congenital atresia or stenosis of the portal vein, extrinsic compression (tumors), and portal vein thrombosis (PVT). In these disorders, the obstruction in the prehepatic portal venous system leads to an increased portal venous pressure [16].

The isolated obstruction of the SV (mainly due to thrombosis) usually results in left-sided PH (sparing the superior mesenteric district). In this rare clinical condition, the blood flows retrogradely through the short and posterior gastric veins and the gastroepiploic veins, leading to the formation of isolated gastric varices. The most common causes of SV occlusion are pancreatic diseases, such as pancreatic cancer, pancreatitis, or a pseudocyst. Although very rare in children, it should be considered in the presence of isolated gastric bleeding with normal liver function and unexplained splenomegaly. The diagnosis may be difficult, and splenectomy represents the treatment of choice in symptomatic patients [17–19].

PVT is the most prevalent cause of extrahepatic portal vein obstruction (EHPVO), and is the major cause of noncirrhotic PH in children. Conversely, congenital abnormalities, such as portal vein stenosis, atresia, and agenesis, are relatively uncommon.

The etiology of PVT remains obscure in approximately 50% of the cases, whereas known etiologies include umbilical vein catheterization, omphalitis/umbilical sepsis, thrombophilia (acquired, hereditary), myeloproliferative disorders, surgery (splenectomy, liver transplantation), dehydration,

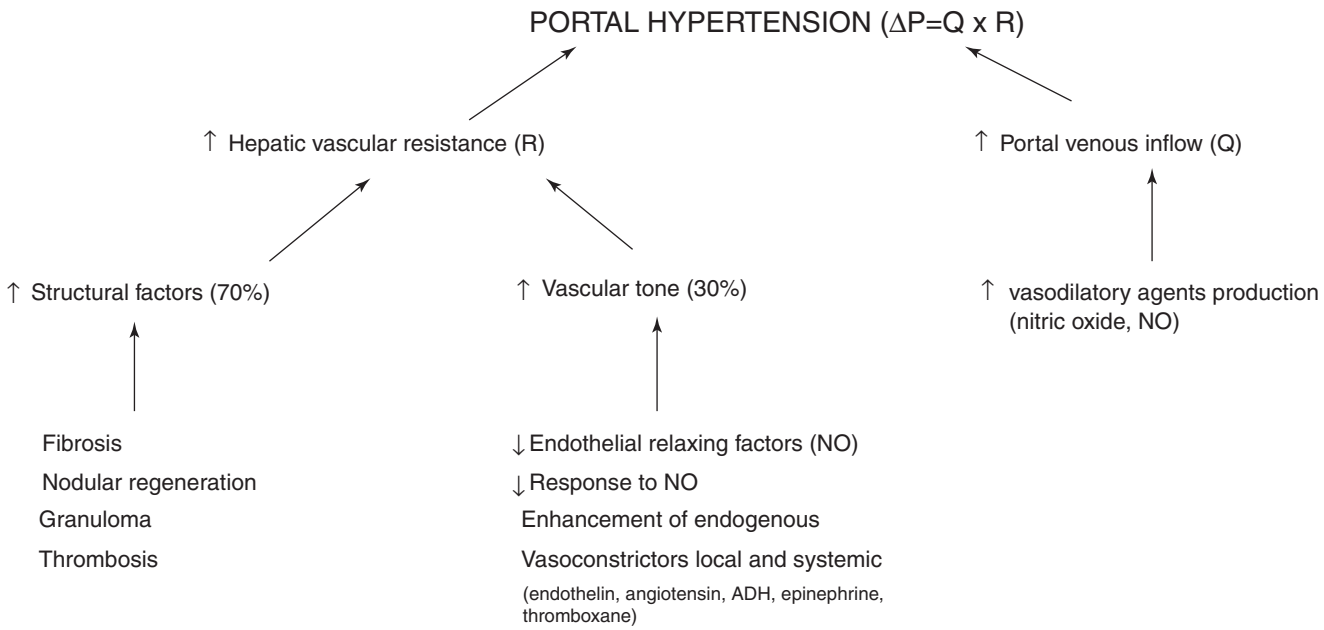

Fig. 71.2 Portal hypertension development according to Ohm's law

Table 71.1 Classification and etiology of portal hypertension

Prehepatic
Portal vein thrombosis
Congenital stenosis or extrinsic compression of the portal vein
Splenic vein thrombosis
Artero-venous fistulae
Intrahepatic presinusoidal
Congenital hepatic fibrosis
Chronic viral hepatitis (HBV and HCV)
Primary biliary cirrhosis
Myeloproliferative diseases (Hodgkin's disease, leukemia)
Focal nodular hyperplasia
Idiopathic portal hypertension (IPH)/non-cirrhotic portal fibrosis (NCFP)/hepatoportal sclerosis
Granulomatous diseases (schistosomiasis, sarcoidosis, tuberculosis)
Amyloidosis
Gaucher's disease
Polycystic liver disease
Infiltration of liver hilum (independent of cause)
Benign and malignant neoplasms
Toxins and drugs (arsenic, vinyl chloride monomer poisoning, methotrexate, 6-mercaptopurine)
Peliosis hepatis
Rendu–Osler–Weber syndrome
Chronic hepatitis
Intrahepatic sinusoidal
Liver cirrhosis (independent of cause)
Wilson's disease
Hemochromatosis
Storage diseases (fatty liver, glycogenosis type III, Niemann–Pick disease, α1-antitrypsin deficiency)
Acute hepatitis (viral and autoimmune)
Hypervitaminosis A
Intrahepatic postsinusoidal
Veno-occlusive disease (VOD)
Hepatic vein thrombosis (Budd–Chiari syndrome)
Posthepatic
Inferior vena cava obstruction (thrombosis, neoplasms)
Right heart failure
Constrictive pericarditis
Tricuspid valve diseases

HBV hepatitis B virus, *HCV* hepatitis C virus

and multiple exchange transfusions in the neonatal period [20–22].

In a multicenter Italian study including 187 pediatric patients diagnosed with EHPVO, it was shown that the condition is strictly associated with a neonatal disorder. The mean age at diagnosis was 4 years; 59% were born preterm; 65% had a history of umbilical catheterization; 82% had associated illnesses, such as complications of prematurity (43.5%), cardiac malformations (7.5%), noncardiac malformations (8.5%), deep infections (7%), and hematological disorders (5.5%). The patients were diagnosed upon detection of splenomegaly (39.5%), after an episode of GI bleeding (36.6%), because of hypersplenism (5.2%), by chance in the context of other investigations (16.3%; personal data, unpublished).

The pathogenesis of PH in EHPVO is closely related to the portal vein obstruction, which causes an increased vascular resistance in the portal venous system. Initially, the occlusion of the portal vein by thrombus formation is followed by compensatory vasodilatation of the hepatic artery buffering the need for blood supply to the liver. Eventually, collateral venous vessels bypassing the thrombus develop and constitute the so-called cavernomatous transformation or portal cavernoma. Part of these collaterals may reperfuse the liver, whereas the majority contributes to the porto-systemic shunting developing at various levels in the portal system. Liver tests are usually normal since there is no parenchymal disease apart from mild vascular changes, such as portal venous dilatation and sclerosis.

The management of EHPVO is mainly directed to the treatment of PH complications through medical and endoscopic means. Despite their efficacy in obliterating esophageal varices (EV), endoscopic methods have no effect on portal pressure. Conversely, surgical procedures may decompress the portal venous system and normalize the portal vein pressure. Possible indications for surgical treatment include acute variceal bleeding that cannot be controlled by endoscopic means, persistent EV formation, massive symptomatic splenomegaly, growth retardation, and symptomatic portal biliopathy [23, 24]. EHPVO is dealt with more extensively in the non-cirrhotic PH section.

Posthepatic causes of increased resistance to flow are those related to vascular and/or cardiac diseases, including thrombosis/stenosis of the hepatic veins or the atrio-caval junction; any condition increasing the right atrial pressure, such as constrictive pericarditis; severe tricuspidal regurgitation; and right-sided cardiac failure. The postsurgical status of some congenital cardiac malformations, such as the Fontan circulation, results in increased central venous pressure and increased resistance to liver outflow [25]. Unlike prehepatic PH, in which liver function remains often normal overtime, in posthepatic PH, the liver blood stagnation may compromise liver function leading to cirrhosis [26].

Budd–Chiari syndrome (BCS) is one of the most common causes of posthepatic PH both in adults and children. BCS is characterized by hepatic venous outflow obstruction at any level from the small hepatic veins to the atrio-caval junction, regardless of the cause of obstruction. The acute increase of vascular resistance secondary to the hepatic venous outflow obstruction causes the sudden appearance of PH, whereas the chronic status may lead to cirrhosis [27].

A wide variety of predisposing causes may determine the onset of the BCS, including congenital or acquired webs of the IVC and thrombotic, inflammatory, or neoplastic processes.

BCS is relatively rare in children. In studies including patients younger than 10 years with PH, the percentage of cases of BCS accounted for 1–7% of all, although in some

areas such as Africa, India, and China, the rate of pediatric BCS may raise up to 16% [28, 29].

The presentation of BCS can be acute, chronic, or fulminant. In the early course of the disease, it may be asymptomatic and accompanied by normal liver tests. Eventually, the hepatic venous outflow obstruction may lead to hepatic dysfunction associated with abdominal pain, ascites, and hepatosplenomegaly.

Due to its rarity, BCS in children is often diagnosed with some delay. The stage of the disease at diagnosis influences the management strategy, and an early diagnosis offers the best chance of cure without major surgery [30].

The management of BCS in pediatric patients may include the use of anticoagulation, thrombolytic therapy and angioplasty with or without stenting, transjugular intrahepatic porto-systemic shunts (TIPS), and, rarely, surgical porto-systemic shunts, the latter carrying high risk of thrombotic obstruction. Some patients may end up with end-stage liver disease and require transplantation.

Intrahepatic Causes of PH

Intrahepatic causes of increased vascular resistance have a more various and complicated pathogenesis compared to the extrahepatic forms, and can be further subdivided, according to the relation with the sinusoidal bed, into three subgroups: presinusoidal, sinusoidal, and postsinusoidal.

Presinusoidal venous block can be caused by many conditions, as detailed in Table 71.1. These disorders cause an elevated portal venous pressure, which cannot be detected by the hepatic vein catheter study, since wedged hepatic venous pressure (WHVP) reflects that of the sinusoids that are distal to the lesion, and therefore have normal blood pressure in this condition. Thus, the only useful technique to gather information on the degree of presinusoidal PH is that of the direct measurement of portal or splenic pulp pressure (Table 71.2).

Schistosomiasis is one of the leading causes of PH in the developing countries. Liver involvement due to schistosomiasis is caused by one of the two trematode flukes *Schistosoma mansoni* and *japonicum*. While the former is seen predominantly in Africa and South America, the latter is common in eastern Asia, especially mainland China [31]. The pathogenesis of liver disease here is secondary to entrapment of eggs in the portal venules that cause granulomatous inflammation leading to fibrosis and, in 4–8% of cases, presinusoidal PH. Portal tract inflammation results from the host response to the parasitic egg in the hepatic venule. The natural history of PH in this condition is closely related to the number of eggs deposited in the liver [32, 33].

Sinusoidal obstruction is mainly due to cirrhosis. It is marked by an increase of HVPG, normal free hepatic venous pressure (FHVP), and raised WHVP (Table 71.2). In sinusoidal PH, WHVP is equal to portal venous pressure because disrupted intersinusoidal communications diminish compressibility and compliance of the sinusoids, allowing direct transmission of portal pressure to the WHVP [34, 35]. In cirrhosis, the increase of vascular resistance occurs at the level of the hepatic microcirculation (sinusoids), and it is secondary to both a *mechanical* and a *dynamic factor*. The *mechanical factor* is represented by the hepatic architectural derangement, and is characterized by hepatocyte swelling, hyperplasia, portal tract inflammation, and fibrosis in response to liver injury. Besides, collagen deposition in the space of Disse may contribute to increased intrahepatic resistance [36]. The *dynamic factor* is represented by the active contraction of myofibroblasts and vascular smooth-muscle cells of the intrahepatic veins, and it may be modified by endogenous molecules and pharmacological agents, which affect the intrahepatic vascular resistance. Factors that increase the hepatic vascular resistance include endothelin-1 (ET-1), the alpha-adrenergic stimulus, and angiotensin II. Those decreasing hepatic vascular resistance include NO, prostacyclin, and vasodilating drugs (e.g., organic nitrates, adrenolytics, calcium channel blockers) [15, 37, 38].

Among these endogenous factors, ET-1 and NO play a key role in regulating the hepatic vascular resistance. ET-1 is a powerful vasoconstrictor synthesized by sinusoidal endothelial cells that has been implicated in the increased hepatic vascular resistance of cirrhosis, and in the development of liver fibrosis. NO is a powerful vasodilator substance that is also synthesized by sinusoidal endothelial cells. In the cirrhotic liver, the production of NO is decreased, whereas that of ET-1, it is increased. The result of these changes is a net vasoconstrictive effect that, in cirrhosis, accounts for

Table 71.2 Hepatic venous pressures according to the pathophysiology of portal hypertension

Etiology of PH		ISP	PVP	RAP	WHVP	FHVP	HVPG
Prehepatic		↑↑	↑↑	N	N	N	N
Intrahepatic	Presinusoidal	↑↑	↑↑	N	N or ↑	N	N or ↑
	Sinusoidal	↑↑	↑↑	N	↑↑	N	↑↑
	Postsinusoidal	↑↑	↑↑	N	↑↑	N	↑↑
Posthepatic		↑↑	↑↑	N or ↑	↑↑	↑↑	N or ↑

ISP intrasplenic pressure, *PVP* portal vein pressure, *RAP* right atrial pressure, *WHVP* wedged hepatic venous pressure, *FHVP* free hepatic venous pressure, *HVPG* hepatic venous pressure gradient (difference between WHVP and FHVP), ↑↑ severe increase, ↑ mild increase, *N* normal

approximately 20–30% of the increased intrahepatic resistance [39–41].

Another dynamic factor that can lead to an increase of intrahepatic vascular resistance is mediated by stellate cells. Hepatic stellate cells (HSCs) are located in the perisinusoidal space of Disse, behind the endothelial barrier, resulting in 5–8% of all human liver cells and 13% of sinusoidal cells. HSCs are involved in vitamin A storage and the synthesis of extracellular matrix components, matrix-degrading metalloproteinase, cytokines, and growth factors [42].

HSCs have the capacity to contract or relax in response to vasoactive mediators, such as ET-1 and NO, therefore having a crucial role in controlling intrahepatic vascular resistance and blood flow at sinusoidal level. Indeed, stellate cells become "activated" in response to acute or chronic noxae damaging the liver parenchyma, acquiring a myofibroblast-like phenotype. During HSC activation, their production of extracellular matrix changes qualitatively and quantitatively, leading to an increase of intravascular resistance.

In summary, in cirrhosis, the increased intrahepatic vascular resistance consists of two main components. The "mechanical factor" is fixed and caused by the structural changes, which occur in patients with chronic liver disease mainly in the form of fibrosis and nodule formation [43, 44]. The "dynamic factor" is variable and caused by endogenous mediators (ET-1 and NO) as well as HSC activation. The main target of the management of PH is represented by medical therapy directed against the "dynamic factor" to decrease the intrahepatic vascular resistance [45, 46].

Postsinusoidal obstruction includes right-sided heart failure, IVC obstruction, small venule BCS, and veno-occlusive disease (VOD). In this setting, WHVP is elevated, whereas HVPG and FHVP can be either normal or elevated, depending on the site of obstruction, intrahepatic postsinusoidal or posthepatic, respectively (Table 71.2). Hepatic VOD is a clinical syndrome occurring early after bone marrow transplantation (BMT) as a result of liver damage by pretransplant conditioning, or chemotherapy for solid tumors. Its incidence in the pediatric BMT population is between 22 and 28%, with an associated mortality of up to 47% [47, 48]. The pathologic injury initiates in zone 3 of the liver acinus with subendothelial edema of hepatic venules, fibrin deposition, microthrombosis, venular narrowing, and sclerosis, followed by hepatocyte necrosis [49]. The result is a postsinusoidal increased resistance to hepatic venous outflow resulting in acute PH and, in some cases, multiorgan failure [50–52].

Other Pathogenetic Mechanisms of PH

In some conditions, PH can be caused by the increase of portal venous inflow itself. In patients with an artero-venous communications between the splanchnic arteries and the portal venous system, an *artero-portal fistula* (APF), the portal flow is markedly increased and arterialized, with the consequent development of presinusoidal PH. APF can be acquired or congenital, but the most common causes are hepatic trauma and liver biopsy, and can be asymptomatic or manifest with PH. Long-standing APF can lead to severe PH characterized by arterial Doppler signal in the portal vein, reversal of the portal flow, and thickening/narrowing of the extrahepatic portal vein. In this setting, radiological procedures represent the best treatment option to close the artero-venous fistula and restore a normal portal vein flow, although, in the congenital forms, the fistula often reappears through the development of new spontaneous shunt formation [53].

In 1898 Banti described a disorder characterized by splenomegaly and hypersplenism, resulting in PH and anemia in the absence of hematological and liver disease. The actual existence of the condition has been questioned for a long time due to the lack of explanation for the development of splenomegaly, hypersplenism, and PH in these patients. Nowadays, Banti's syndrome is considered the result of microscopic changes of the portal tract that were not detected at the early stages of its clinical description and corresponding to a group of diseases causing non-cirrhotic PH. *Hepatoportal sclerosis* (HS) is one of the rare disorders characterized by sclerosis of the intrahepatic portal veins resulting in non-cirrhotic PH. HS in children is uncommon but probably underestimated, and only few case reports have been published so far. The cornerstone of the diagnosis of HS is the histology, characterized by portal fibrosis without evidence of either cirrhosis or nodule formation; portal fibrosis is responsible for the increase in the intrahepatic vascular resistance and PH. Nevertheless, the mechanisms leading to portal fibrosis and, in general, the entire phenotype of HS are still not well known [54]. Yilmaz reported on 12 pediatric patients with non-cirrhotic PH. On histology, all patients had HS or intimal fibrous thickening of portal vein and periportal fibrosis, acinar transformation, and regenerative nodules not surrounded by fibrous septa. In some of them, there were also signs compatible with cholestatic disease, including neoductular reaction in seven, mild cholangitis in one, and canalicular bile pigment in one [55].

Systemic Hemodynamic Changes in Portal Hypertension

Increased resistance to portal blood flow is likely to be the *primum movens* in the development of PH; however, a variety of hemodynamic changes contribute to amplify the increased portal venous pressure observed in patients with chronic liver disease.

The hyperdynamic syndrome was first described in the 1950s, when some physicians observed that patients with

cirrhosis often showed "warm extremities, cutaneous vascular spiders, wide pulse pressure and capillary pulsations in the nail beds." In 1953, Kowalski and Abelmann published the first study which demonstrated an increase in cardiac output and a decrease in peripheral vascular resistance in patients with alcohol-induced cirrhosis [56]. The recognition of the dangerous effect of this syndrome on multiple organs, though, was achieved only several years later [57].

Vasodilatation plays a key role in the development of the hemodynamic changes. The hyperdynamic syndrome should be better called "progressive vasodilatatory syndrome," because vasodilatation is the main factor that brings about all the vascular changes and finally the multiorgan involvement seen in cirrhosis [58]. A major step ahead in this field was accomplished in the 1990s, when researchers discovered that NO was responsible for the vasodilatation and, in turn, of the multiple organ malfunctions characterizing the hyperdynamic circulation [59].

Both clinical studies and animal models have demonstrated and explained the hemodynamic events that occur in PH, but, since they have not been performed in children, the findings should be interpreted with caution (Fig. 71.2).

Splanchnic Circulation

Vasodilatation of the splanchnic circulation is a process mediated by humoral vasodilatatory agents, and it is probably the initial signal triggering the hyperdynamic systemic circulation. Splanchnic vasodilatation causes, as a consequence, an increased portal venous blood inflow, contributing to the maintenance and the aggravation of PH [57, 60]. The result of this significant vasodilatation is that a large proportion of circulating blood volume remains confined to the splanchnic system, with a subsequent reduction of central blood volume. This process is called the "forward flow" theory and provides a rationale for the use of vasoconstrictors in adult patients with PH [12].

Systemic Circulation

Splanchnic vasodilatation is associated with changes in the systemic circulation, such as a decrease of arterial pressure, that is, consequence of the decreased central blood volume and peripheral resistance in various organs [61]. Compensatory mechanisms include the activation of baro- and volume receptors as well as the production of neurohormonal substances leading to sodium and water retention, with plasma volume expansion and increase in cardiac output [62].

The cardiac response is directly related to splanchnic vasodilatation and plasma volume expansion, together with an increased venous return that is mostly due to the formation of porto-systemic shunts. Although vasodilatation is essential as the initiating factor, no hyperdynamic circulation occurs without expansion of plasma volume and porto-systemic shunting [63]. The former is due to renal sodium retention, which has been shown to precede the increase in cardiac output, and can be prevented or reversed by sodium restriction and administration of spironolactone. The latter is characterized by the development of new veins (called collateral vessels) bypassing the liver and decompressing the portal venous system. These veins directly connect the portal blood vessels to veins that divert the blood away from the liver into the systemic circulation. The drawback in this compensatory process is that substances (such as ammonia and toxins) that are normally removed from the blood by the liver pass directly into the systemic circulation, and have adverse effects in other organs [64].

Collateral vessels tend to develop at the lower end of the esophagus and at the upper part of the stomach (Fig. 71.1). Here, the vessels enlarge and become full of twists and turns, becoming varicose veins in the esophagus varices (EV) or stomach (gastric varices). Other collateral vessels may develop on the abdominal wall and in the rectum. These vessels are prone to rupture, leading to GI bleeding.

Lung Circulation

PH and liver shunting may also affect the lungs, resulting in the development of hepatopulmonary syndrome (HPS) and portopulmonary hypertension (PPH); these conditions are characterized by hypoxia due to pulmonary artero-venous shunts and pulmonary hypertension, respectively [65]. Although the intrinsic mechanism triggering these complications is not fully known, the major role seems to be played by molecules active on the pulmonary endothelium (including NO and carbon monoxide) that can cause either condition [66, 67].

Renal Circulation

Renal circulation is affected indirectly by the hyperdynamic state. To balance the progressive systemic vasodilatation, the kidney responds to a perceived hypovolemia by retaining sodium and water. The relative hypovolemia results from an increase of the vascular compartment caused by vasodilatation, leading to the activation of vasoconstrictive and volume-retaining neurohumoral substances that perpetuate sodium and water retention [68]. These compensatory mechanisms

include the activation of renin–angiotensin–aldosterone system and antidiuretic hormone secretion. In the early course of the disease, the intravascular volume and the cardiac output increase to maintain the arterial perfusion pressure [69]. With the progression of the disease, vasodilatation worsens, and the cardiac output continues to increase up to a maximum, and then it is not enough to maintain the perfusion pressure. At this point, the renal blood flow drops and renal failure develops [70, 71].

The hyperdynamic circulation should not be considered a complication of cirrhosis but a complication of PH. In fact, it was observed also in non-cirrhotic subjects and confirmed in different experimental models of PH [57, 59].

Table 71.3 Clinical evaluation and investigations useful to recognize patients with suspected portal hypertension

Step	Aim
Clinical history	Ask for neonatal umbilical catheterization, episodes of gastrointestinal bleeding, results of previous blood tests, investigations for an undefined splenomegaly
Physical examination	Assess liver size and consistency, look for splenomegaly, abdominal venous patterning (site and direction of venous flow), spider nevi and telangiectasias, palmar erythema, ascites, limb edema
Liver function tests	Assess liver function and full blood count for hypersplenism
Ultrasonography and Doppler of the liver	Evaluate liver parenchyma, patency of portal vein and direction of venous blood flow, hepatic vein patency, venous anatomical abnormalities, hepatic artery (patency and abnormalities), porto-systemic shunts, ascites, splenomegaly, renal abnormalities
Upper endoscopy	Assess varices and hypertensive gastropathy
CT scan of the abdomen	Assess liver parenchyma, biliary tree conformation, vascular anatomy, rex recessus patency, and signs of portal hypertensive biliopathy
Measure portal venous pressure (HVPG, WHVP, FHVP)	Evaluate the degree of portal hypertension. Diagnose prehepatic, intrahepatic, posthepatic causes
Liver biopsy	Assess fibrosis/cirrhosis, inflammation, histological pattern

CT computed tomography, *HVPG* hepatic venous pressure gradient, *WHVP* wedged hepatic venous pressure, *FHVP* free hepatic venous pressure

Clinical Manifestation of Portal Hypertension

PH in children has a broad spectrum of clinical manifestations, varying from the occasional finding of splenomegaly discovered during a routine follow-up visit in absence of any symptom to hematemesis and melena due to the rupture of EV (Table 71.3). The main manifestations of PH are GH, ascites, and splenomegaly, but, in a minority of patients, other complications may arise including hepatic encephalopathy (HE), pulmonary vascular disorders, and kidney disease [72].

Gastrointestinal Hemorrhage

GH is defined as bleeding in the digestive tract, and it can be classified as proximal or distal, acute or chronic. Bleeding from the upper digestive tract (esophagus, stomach, and upper portion of the small intestine) causes hematemesis and melena, whereas bleeding from the lower digestive tract (lower portion of the small intestine, large intestine, and rectum) causes dark blood or bright red blood mixed with stool, depending on the proximity to the anal sphincter. GH is mainly related to bleeding from EV and also, in a minority of cases, from portal hypertensive gastropathy, gastric antral vascular ectasia, or gastric, duodenal, peristomal, or rectal varices (Fig. 71.3).

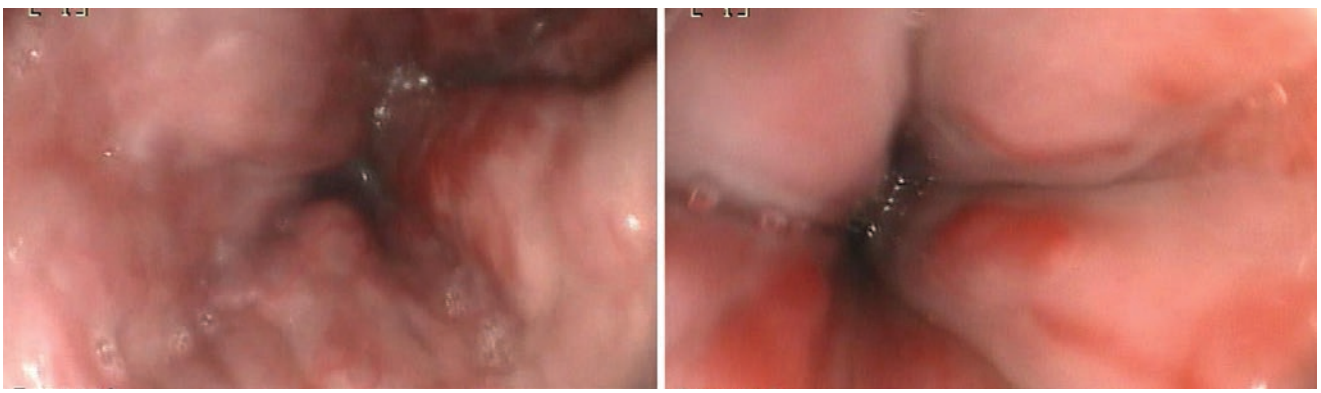

Fig. 71.3 Endoscopic appearance of large esophageal varices with red signs in two children with portal hypertension. (Reprinted from Ref. [73], with permission from Elsevier)

Acute GH is often the first symptom of a long-standing silent liver disease, and therefore it is regarded by patients and carers as a frightening event, giving the impression of imminent death. Although the mortality from GI bleeding in children is lower than in adults, acute GH remains a life-threatening event and requires prompt medical intervention. Chronic bleeding is usually mild and can be discovered since the patient has refractory iron-deficiency anemia and positive fecal occult blood test [73].

The formation of varices and their rupture result from the increased pressure within the vessel as a consequence of PH. When the wall tension exceeds the variceal wall strength, the rupture of the varix occurs, and the patient develops hematemesis and/or melena [74].

Variceal bleeding in children with chronic liver disease often follows an acute upper respiratory tract infection, with the contribution of several factors such as the increased abdominal pressure during coughing or sneezing, the increased cardiac output due to fever, and the erosive effect of nonsteroidal anti-inflammatory drugs used to treat the fever. Gastroesophageal reflux is another factor which may contribute to erosions of varices leading to its rupture and bleeding [75–77].

Hematemesis and melena are the most common presenting symptoms in children with both intrahepatic and extrahepatic PH, and the first episode can be as early as 2 months of age [2, 22, 78–80].

The age at the first bleeding episode is related to the underlying etiology. In children with biliary atresia, the first bleed was described at a mean age of 3 years, while in children with cirrhosis due to cystic fibrosis, it occurred at 11.5 years [74, 81]. In a recent study, 65 children with EHPVO were followed for a median period of time of 8.4 years. Thirty-two (49%) patients presented with bleeding at a median age of 3.8 years (0.5–15.5) and, during the follow-up period, 43 of them (66%) had at least 1 bleeding episode [2]. Triger et al. followed 44 children with EHPVO for a mean follow-up of 8 years. The actuarial probability of bleeding was 49% at age 16 years and 76% at 24 years of age. If the child bled before 12 years of age, the probability of bleeding was higher than in those who had not bled before 12 years of age. Further, there was no evidence of variceal regression over time. These studies do not support the previous hypothesis that variceal bleeding decreased in adolescence due to the development of spontaneous porto-systemic collaterals [2, 82]. In a multicenter Italian study on 187 children with EHPVO, the mean age at diagnosis was 4 years, and the most common symptoms at onset were splenomegaly (40%) and gastrointestinal bleeding (36%). Of 71 patients with an available endoscopy at presentation, 62 (87%) had already developed EV. Development of EHPVO was strictly associated with a neonatal disorder including history of prematurity, neonatal illness, and umbilical venous catheter. Authors concluded that a liver Doppler ultrasound should be performed before discharge from the neonatal unit and at the follow-up to allow an early recognition of the disease and avoid bleeding from EV that are present from the early stages [78]. Since splenomegaly is a very common sign detected in children with PH at the time of GI bleeding, the association between GI bleeding and splenomegaly should be suggestive of PH until proven otherwise [74].

There is no strong evidence supporting the efficacy of any treatment for the prevention of variceal bleeding in children. The administration of nonselective β-blockers (NSBBs), the endoscopic treatment of varices, and the surgical (meso-rex bypass, porto-systemic shunts) and radiological (TIPS) measures to decompress the portal system represent the main therapeutic options for the primary and secondary prophylaxis of bleeding in children with PH [73, 83].

Splenomegaly

Splenomegaly indicates an enlargement of the spleen usually associated with its overactivity, defined hypersplenism, which leads to premature destruction of blood cells. Splenomegaly is due to PH which causes at the beginning only spleen congestion and eventually tissue hyperplasia and fibrosis. The increase in spleen size is followed by an increase in splenic blood flow, which participates in PH actively congesting the portal system [84]. Together with EV, splenomegaly represents the most common finding in children with PH even though, in asymptomatic children, it is often discovered accidentally during a routine physical examination [78].

Despite a big spleen is highly suggestive for PH, many children with liver disease and isolated splenomegaly have often a delayed diagnosis. In clinical practice, splenomegaly accompanied by hypersplenism is considered a sign of hematological disorders, leading to a long hematological follow-up (including bone marrow aspiration and biopsy) before asking consultation to a hepatologist. Due to the large spleen, children with PVT often receive a diagnosis of infectious mononucleosis every time they come to clinical attention because of a viral illness, and PH is disclosed only after a bleeding episode.

Liver function tests and Doppler ultrasound are mandatory in healthy children with splenomegaly and hypersplenism to exclude the presence of EHPVO and avoid worthless procedures [74, 85].

Some studies have tried to identify the best noninvasive method to diagnose the presence of EV in children with PH. Platelet count and splenomegaly are usually considered the most reliable parameter to predict the presence of EV. The clinical prediction rule proposed by Gana has high predictive value (area under the receiver operating characteristic; ROC curve 0.80) and is calculated according to the

following formula: (0.75 × platelets)/(spleen z score + 5) + 2.5 × albumin [86].

Once liver transplantation (LTX) or porto-systemic shunting is performed, splenomegaly and hypersplenism may improve significantly, but sometimes they persist for long, depending on the grade of splenic hyperplasia and fibrosis developed over time [87, 88].

Ascites

Ascites is the accumulation of serous fluid in the peritoneal cavity, and is usually seen in patients with PH due to cirrhosis. Ascites appears when the hydrostatic pressure goes above the osmotic pressure within the hepatic and mesenteric capillaries, and the transfer of fluids from blood vessels to lymphatics overcomes the drainage capacity of the lymphatic system [89].

Ascites should be analyzed to obtain information on its cause and possible complications. The serum ascites albumin gradient (SAAG) is used to classify ascites into portal and non-portal hypertensive etiologies. The SAAG is calculated by subtracting the ascitic fluid albumin level value from the serum albumin value, and the result correlates directly with portal pressure. This phenomenon is the effect of Starling's forces between the fluid of the circulatory system and ascitic fluid, as albumin does not move across membranes easily, because it is a large molecule. Under normal circumstances, the SAAG is ≤1.1 g/dl because serum oncotic pressure (pulling fluid back into circulation) is exactly compensated by the serum hydrostatic pressure (which pushes fluid out of the circulatory system). In presence of PH, there is an increase in the hydrostatic pressure causing more fluid and more albumin to move from the circulation into the peritoneal space with ascites formation. As a consequence, the SAAG increases (≥1.1 g/dl). Thus, a high gradient (SAAG ≥1.1 g/dl) indicates that the ascites is due to PH, whereas a low gradient (SAAG ≤1.1 g/dl) indicates that ascites is not associated with increased portal pressure (Table 71.4). In clinical practice, some conditions may influence the proper value of the SAAG including the sampling of ascites and serum in different states of hydration or the impact of serum globulin concentration [90, 91].

Ascites should also be evaluated for spontaneous bacterial peritonitis (SBP), an ascitic fluid infection without an evident intra-abdominal surgically treatable source. The diagnosis is made by ascitic fluid cell count. The absolute polymorphonuclear cell (PMN) count in the ascitic fluid is calculated by multiplying the total white blood cell count (or total "nucleated cell" count) by the percentage of PMNs in the differential. The diagnosis of SBP is established by an elevated ascitic fluid absolute PMN count (≥250 cells/mm^3), a positive ascitic fluid bacterial culture, and absence of secondary causes of peritonitis [92]. Patients with SBP should receive antibiotic therapy, such as intravenous third-generation cephalosporin, and be considered for liver transplantation.

Table 71.4 Causes of ascites based on serum ascites albumin gradient (*SAAG*)

SAAG ≥1.1 g/dl = portal hypertension	SAAG ≤1.1 g/dl = other causes of ascites
Cirrhosis	Peritoneal lymphoma
Non-cirrhotic liver disease	Serositis
Fulminant hepatic failure	Chronic peritoneal infection
	Tuberculosis
	Other (bacteria, viruses, fungi)
Vascular/heart disease	Low serum colloid osmotic pressure
Portal vein thrombosis	Nephrotic syndrome
Veno-occlusive disease	Protein-losing gastroenteropathy
Budd–Chiari syndrome	Kwashiorkor
IVC obstruction/right heart failure	Hollow organ leak
Benign and malignant neoplasms	Lymphatic
Myxedema	Other (pancreatic, biliary, intestinal)

IVC inferior vena cava, *TBC* tuberculosis

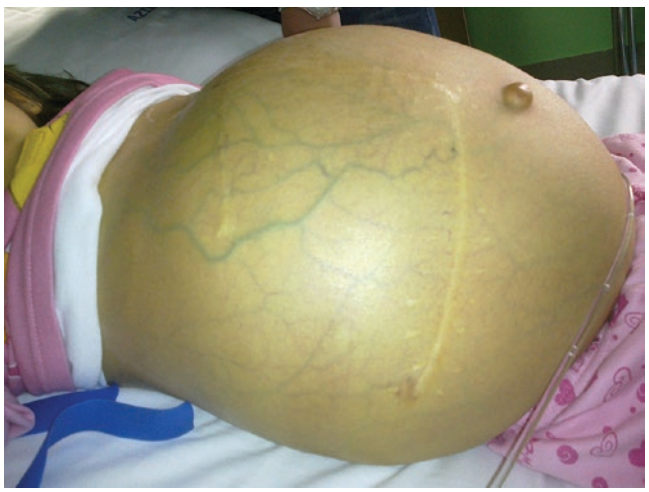

Fig. 71.4 Tense ascites and abdominal venous patterning in a child with biliary atresia, failed Kasai, and end-stage liver disease

Treatment of ascites includes salt and fluid restriction and use of diuretics. Spironolactone is the diuretic of choice as it is an aldosterone antagonist counteracting the endocrine changes of the hyperdynamic circulation, but often there is the need to add a loop diuretic, such as furosemide, which can improve diuresis and counteract hyperkalemia. In children with normal liver synthetic and biliary function, ascites can often be managed with diuretics and occasional paracentesis (Fig. 71.4). If ascites is large and not responding to diuretics, the paracentesis may be utilized safely in children, preferably after albumin infusion [93, 94]. In a prospective

observational study on 32 children with severe ascites, the authors demonstrated that the incidence of post-paracentesis circulatory dysfunction was lower in children who received albumin infusion compared to those who did not (12% vs 67%, $p = 0.003$) [93]. When ascites does not recede and recurs shortly after paracentesis, or when children do not tolerate diuretic therapy due to side effects, the management can take advantage of more aggressive treatment including regular large-volume paracentesis and, if feasible, TIPS. TIPS procedure, although uncommon in children, provided good results in terms of resolution of refractory ascites in both native and transplanted livers [88].

When ascites is accompanied by signs of end-stage liver disease, such as hypoalbuminemia, jaundice, clotting derangement, or SBP, the only effective treatment is liver transplantation. In these cases, albumin infusions can be used along with diuretics, in order to increase the osmotic pressure and facilitate the passage of fluid from the extravascular to the intravascular compartment. In children with end-stage liver disease, ascites can be associated with hyponatremia, which is a risk factor for severe complications and death. Pugliese et al. evaluated the association of pre-transplant variables with the mortality within 90 days following the inclusion on the waiting list of 520 children with cirrhosis. On multivariate analysis, the presence of ascites and serum sodium levels was associated with decreased patient survival while awaiting a liver graft [95].

Chylous ascites is a rare clinical condition marked by an extravasation into the peritoneal cavity of a milky fluid deriving from the mesenteric lymphatic vessels. Usually, it results from major abdominal surgical interventions, such as liver transplantation, during which several lymphatic vessels are inadvertently resected and PH has not yet resolved; nevertheless, chylous ascites can present also in patients with PH due to PVT or congenital portal venous malformation. In this setting, in spite of the absence of strong evidences, the management includes fat-free diet and somatostatin analogues [96].

Pulmonary Complications

Children with PH may develop two rare pulmonary complications: hepatopulmonary syndrome (HPS) and portopulmonary hypertension (PPH). Their relative frequency and risk factors have not been defined, and only isolated cases or small series have been published so far.

The pathogenesis of HPS and PPH remains unclear, but the two conditions arise only in patients with porto-systemic shunting, and therefore the pathogenesis must be related to it. The proposed theories suggest that these disorders result from a combination of the hyperdynamic circulation, the increased cardiac output, the shear injury to the vascular walls, and an imbalance of circulating vasoactive peptides. Abnormal hepatic synthesis of vasoactive peptides, such as EN-1, or impaired hepatic metabolism of intestinally derived endotoxins, cytokines, and neurohormones may result in these substances reaching the pulmonary vascular bed via porto-systemic shunting, directly altering the vessel tone or leading to pulmonary vascular inflammation and remodeling. The resulting pathology is strikingly different in these two disorders, with vasodilatation of pulmonary arterioles and capillaries causing artero-venous shunting in HPS, and intimal fibrosis with endothelial and smooth-muscle cell proliferation leading to increased pulmonary vascular resistance in PPH [97–99].

HPS is defined as intrapulmonary vascular shunting (IPVS), ventilation–perfusion mismatch, and chronic hypoxemia in a setting of liver disease and/or PH. The mechanisms implicated in the development of HPS are likely to include many of the vasoactive substances involved in the genesis of the hyperdynamic circulation, including NO and EN-1 [100–102]. Porto-systemic shunting plays a key role in the pathogenesis of the HPS; in fact, HPS has been described also in patients with congenital porto-systemic shunting and no liver disease (i.e., the Abernethy malformation).

From the clinical point of view, HPS is characterized by shortness of breath, exercise intolerance, and digital clubbing. Since the disease is often subtle and progresses slowly, in the early stages, it can be easily overlooked and become overt only when advanced. Patients with PH should be screened for HPS by measuring the transcutaneous oxygen saturation and, if <96%, by performing further investigations to assess the real presence of IPVS. The two main procedures to confirm the presence of IPVS are the echocardiography with agitated saline and the macroaggregated albumin scan. The former is simple and sensitive in both symptomatic and asymptomatic children. The latter may be used to quantify the degree of shunting, which can be useful in clinical decision-making and to test the progression of HPS over time [103–105].

Liver transplantation represents the only effective treatment option for children with HPS. Al-Hussaini et al. reported a study on 18 children with HPS over 14 years. Fourteen underwent LTX with resolution of HPS in 13. Six developed vascular or biliary complications and four died (two before transplantation) [106]. Interestingly, the need of oxygen may temporarily persist even many days after LTX. In Warner's study all 15 children (100%) with HPS needed supplementary oxygen after LT for a median of 12 days and with a range of 2–139 days. The authors reported a statistically significant association between a Hb of >130 g/L on the day of LTX with a longer length of supplementary oxygen requirement ($P = 0.005$). Therefore, they concluded that the longer the children with HPS have to wait for a LT, the more polycythemic they will become, and the longer they will need supplementary O2 after LT [103].

PPH is defined as an elevation of the mean pulmonary arterial pressure and increased vascular resistance caused by a pulmonary arteriopathy, in the setting of PH and in the absence of underlying cardiopulmonary disease [105]. The pathophysiology of this disorder is still unclear; however, it seems to be related to a decreased hepatic clearance and porto-systemic shunting of biochemical mediators in the setting of liver dysfunction and PH. PPH produces characteristic histological changes in the pulmonary vasculature that have been well documented on autoptic samples [107]. Although there are scant data in children, it has been shown that PPH can develop in patients with cirrhotic and non-cirrhotic causes of PH such as biliary atresia, PVT, focal nodular hyperplasia, and congenital hepatic fibrosis [108]. From the clinical point of view, PPH presents most commonly with exertional dyspnea, fatigue, palpitations, and syncope or chest pain. The symptoms are often subtle at onset, so that a high index of suspicion is required to diagnose PPH in asymptomatic patients before they develop severe and irreversible pulmonary hypertension [107]. Echocardiography with Doppler flow is considered the most useful screening modality in patients with suspected pulmonary hypertension, although limited by the fact that it relies on the presence of tricuspidal regurgitation, it measures systolic rather than mean pressure in the right ventricle and the measurement is approximate. Chest X-ray and electrocardiogram (ECG) definitely lack sensitivity. If there are indirect signs of pulmonary hypertension at ECG, further evaluation should include a right heart catheter study and, in some cases, high-resolution computed tomography (CT) of the chest and ventilation–perfusion lung scanning [104].

If the diagnosis of PPH is made early before the development of irreversible pulmonary vasculopathy, LTX can be successfully performed and may reverse the process. Conversely, once PPH is advanced (with a mean pulmonary pressure >35 mmHg) and associated with right-sided heart failure, LTX becomes unfeasible because of the functionally obstructed liver outflow that leads to graft failure and death in at least 50% of cases. More recently, it has been shown that the perioperative use of inhaled and intravenous pulmonary vasodilators (NO and epoprostenol) as well as oral drugs (sildenafil and bosentan) can remarkably reduce the pulmonary pressure to a safe level, allowing to perform LTX [108]. The goals of treatment are, therefore, to lower the mean pulmonary arterial pressure and move the patient from high risk to a safer ground for transplantation. However, if there is no response or the pressures remain very high, the only viable option is a combined lung–liver transplant.

Other Major Complications of Portal Hypertension

An abnormal *abdominal venous patterning* can be seen in children with PH, in whom a prominent subcutaneous vascular pattern develops as part of spontaneous porto-collateral shunting (Fig. 71.4). This is the result of the attempt at decompressing the portal venous system through the umbilical vein recanalization that leads to periumbilical collaterals. Although less common than in adults, both umbilical venous shunts and rectal varices can be observed in children with long-standing PH, whereas in children with PH and an intestinal stoma (i.e., in short bowel syndrome associated with liver disease), stomal varices often occur and represent a site of low resistance and bleeding [109].

Hepatorenal syndrome (HRS) is defined as a functional renal failure in patients with liver disease and PH, and it constitutes the climax of the systemic circulatory changes associated with PH [91]. In pediatric patients, HRS is rare, probably due to the relatively short time that cirrhotic children spend on the transplantation waiting list. Two types of HRS have been identified. Type 1 HRS is an acute and rapidly progressive form that often develops after a precipitating factor such as GI bleeding or SBP. Type 2 HRS is a slowly progressive form of renal failure that often occurs without a sudden trigger in the setting of chronic and refractory ascites HRS arises from severe vasoconstriction of the renal circulation to compensate for the characteristic circulatory imbalance of advanced cirrhosis. This leads to an increased renal arterial resistance which in turn causes renal hypoperfusion and arterial hypotension. The small volume of the produced ultrafiltrate is then reabsorbed almost completely in the proximal tubule, whereas no solutes (such as sodium) flow to Henle's loop with nearly no hyperosmolar natriuresis, activation of adiuretin–vasopressin, and reduced urine output. As a result, standard diuretic treatment has little effect on diuresis [110]. The criteria to diagnose HRS are difficult to be applied in young children because of the lack of pediatric data. HRS is a potentially reversible condition, but its natural prognosis is poor. Various vasoconstrictors are useful in the treatment of HRS, and terlipressin is the first choice [111]. In the pediatric setting, the experience is little. In a report, four children with end-stage liver disease received terlipressin treatment for renal failure compatible with HRS type 1 in three and type 2 in one. All four responded well and no side effects were reported [112]. Liver transplantation is the ultimate treatment for HRS, ensuring full recovery and long-term survival, and thus it remains the principal tool both in adults and children.

Hepatic encephalopathy (HE) refers to a variety of reversible neurological abnormalities reported in patients with cirrhosis and PH associated with anatomical and functional porto-systemic shunting. In children, HE can be subtle, and the condition seems to appear at a later stage of liver disease and be difficult to diagnose, especially in ill infants. Disturbed consciousness (including coma), personality changes, intellectual deterioration, and speech and motor dysfunction are common in older children with HE. These symptoms usually have a sudden onset and a rapid reversibility suggesting they are of metabolic origin [113].

Protein diet restriction, cleansing enemas, oral antibiotic, and lactulose are the most effective medical options to prevent and treat HE [114, 115].

Non-cirrhotic Portal Hypertension

Non-cirrhotic PH (NCPH) is a heterogeneous group of liver disorders characterized by PH in absence of cirrhosis and with normal or only mildly elevated HVPG values [116].

They are of crucial importance in pediatric hepatology since, while the majority of children with cirrhotic PH are treated with liver transplantation successfully in the early years of the life, those with NCPH do not have any indication for LTX and are managed and followed up for a long time up to adult age [73].

In relation to the site of increased vascular resistance to blood flow, such disorders may be classified as prehepatic, hepatic, and posthepatic. Among presinusoidal NCPH disorders, non-cirrhotic portal fibrosis (NCPF) and EHPVO represent two different entities in whom features of PH are not associated with significant parenchymal dysfunction [116].

NCPF is mostly a disorder of young adults or middle-aged women, whereas non-cirrhotic EHPVO is reported both in infancy and in older children. Recently, it has been proposed the so-called unifying hypothesis, providing a common explanation to the pathogenesis of both NCPF and EHPVO, and focusing on thrombotic events affecting the portal branches. The authors hypothesize that a major thrombotic event occurring at early ages and involving the portal trunk results in EHPVO, whereas repeated microthrombotic events occurring later in life and affecting the small or medium branches of the portal vein would lead to NCPF [116]. In this session, we focus on such two disorders. NCPF is a rather unknown liver disorder in children, whereas EHPVO represents the most common cause of NCPH in the pediatric population.

Obliterative Portal Venopathy (OPV)

The clinical pattern of presentation of OPV is that of PH in the absence of an evident cause, such as liver fibrosis/cirrhosis or vascular obstruction. Liver disorders named idiopathic PH (IPH), idiopathic non-cirrhotic portal hypertension (INCPH), hepatoportal sclerosis (HS), and non-cirrhotic portal fibrosis (NCPF) are thought to be the common consequences of lesions of the intrahepatic branches of the portal vein and correspond to various stages of the same disease, called OPV [116, 117].

On histology, the main features include phlebosclerosis, fibroelastosis, periportal and perisinusoidal fibrosis, aberrant vessels in portal tract (portal angiomatosis) with preserved lobular architecture, and differential atrophy. The main portal vein branch is dilated, with thick sclerosed walls, along with thrombosis in the medium and small portal vein branches, giving a picture of "obliterative portal venopathy" [118, 119]. However, in children, these features are often subtle and the condition may be overlooked.

The etiology of OPV is undefined, but the attention has been brought to various factors that may trigger autoimmunity or endotoxin-mediated injury leading to vascular abnormalities, which cause presinusoidal block to the portal venous flow. The interest has been pointed particularly on lack of hygienic conditions, which would support the role of infections as trigger of the disease, and prothrombotic disorders, which would support the association with an underlying prothrombotic state [120].

With regard to NCPF, the diagnosis is based on clinical evidence of PH without liver dysfunction and a histology with no significant fibrosis. The Asian Pacific Association for the Study of the Liver (APASL) has proposed some criteria for the diagnosis of NCPF in adults [121]. Recently, Schouten et al. redefined five criteria including:

1. Any one of the following clinical signs of PH: splenomegaly, EV, ascites, raised HVPG, and evidence of porto-systemic collaterals
2. Exclusion of cirrhosis on liver biopsy
3. Exclusion of known causes of chronic liver disease causing cirrhotic or non-cirrhotic PH
4. Exclusion of common conditions causing NCPH
5. Patent portal and hepatic veins

All five criteria must be fulfilled to diagnose NCPF [122].

Of interest, characteristic histological lesions are unevenly distributed, vary greatly in their severity, and are not all necessarily present in an individual case, making the diagnosis of OPV often challenging. In a recent study on idiopathic NCPH, Guido and co-authors proposed a new nomenclature and definition of histological lesions including:

(a) Portal vein stenosis: defined as incomplete or complete obliteration of the portal vein branches with or without thickening of the wall
(b) Herniated portal vein: defined as a portal vein from the portal tract directly abutting periportal parenchyma

(c) Hypervascularized portal tract: defined as multiple thin-walled vascular spaces in the portal tract
(d) Periportal abnormal vessels: defined as single or multiple thin-walled vascular spaces of different caliber outside, but in close contact with, the portal tract

The presence of at least one or more of the above lesions is required to diagnose a patient with OPV [119]. In adults, the most common symptoms at onset include bleeding from varix rupture, splenomegaly with or without hypersplenism, and ascites in 10–34% of the cases. On physical examination, the liver may be normal, enlarged, or slightly shrunken, whereas the clinical signs of chronic liver disease are absent [123]. Liver function tests are usually normal in NCPF, but derangements in liver enzymes, prothrombin time, and albumin are seen in a small proportion of adult patients [124]. Hemodynamic studies showed that the increased vascular resistance in NCPF is pre- and perisinusoidal. HVPG is normal or slightly elevated (median 7 mmHg) in this condition [125]. In adults, the natural history of NCPF seems benign, with an overall good outcome. However, in the long term, 30–33% of the adults develop liver atrophy and possible decompensation, develop PVT and HPS, and, sometimes, need LTX [126, 127].

In childhood, OPV is an uncommon cause of PH, but, since the awareness among pediatric specialists is still low, this condition is probably underdiagnosed. The published experiences in this disease are scarce, and they come mainly from Asiatic regions.

Girard et al. reported a child with Adams–Oliver syndrome and HS. They hypothesized that a vascular anomaly and thrombosis may be the etiology for this condition based on the fact that the patient had PVT and factor V Leiden mutation. However, the same association has not been described in other children so far [128].

Prolonged exposure to several medications and toxins has also been proposed as the possible cause. Indian studies on children with PH showed that, among 134 cases, 29 (22%) were due to NCPF. The authors carried out a sociodemographic study that found a significant association with residency in arsenic-affected areas [129]. Toxins can surely lead to liver injury, but a strong association between arsenic intoxication and development of NCPF has not been proven.

Poddar published the experience on 388 Indian children with PH (median age 11 years). Eleven of them (3%) were diagnosed with NCPF. Variceal bleeding, splenomegaly, and a lump in the left upper abdomen were the most common symptoms at onset [130]. Cantez described 12 children (median age 13.5 years) with a histological diagnosis of HS. Four patients had splenomegaly, three had EV, and one had developed HPS and had been transplanted, whereas the others did not show symptoms of PH [131]. Yilmaz reported on 12 children who had a diagnosis of HS, but some of them had also cholestatic features on histology. The authors concluded that cholestatic features noticed in histopathological evaluation may represent a variant group in the spectrum of this disease [55]. A special mention is needed for HIV-related NCPF. The condition occurs predominantly in males (50–100%) and homosexuals (50–75%), with a prolonged infection (median 11.5 years, range 7–15 years). It is not known whether the development of NCPF is related to the infection or rather to the antiretroviral treatment. A recent study described a 10-year-old HIV-infected girl who was on antiretroviral therapy. She had splenomegaly and presented with a massive bleeding from EV rupture. The liver biopsy showed features compatible with NCPF. HVPG was normal. She was managed successfully by treatment with β-blockers and endoscopic variceal eradication [132].

Of note, symptoms of PH may be absent at onset. In a study on 48 children with OPV, 9/48 (19%) did not have symptoms of PH [117]. Similarly, in a multicenter Italian study on 49 children with OPV, features of PH were recorded in 32 patients (65%, 25 with esophageal varices, 7 with splenomegaly and hypersplenism alone); conversely, no symptoms of PH were reported in 17 patients (35%) (personal, unpublished data). Further studies are warranted to best define the real frequency of OPV in children, and to understand the underlying pathogenetic mechanisms leading to PH, in order to define the best therapeutic strategy.

Extrahepatic Portal Vein Obstruction

EHPVO is defined by the obstruction of the extrahepatic portal vein with or without the involvement of the intrahepatic portal veins. The Baveno VI consensus definition excludes cirrhosis and other liver diseases such as IPH, and emphasizes that EHPVO in these settings is a different entity. Isolated occlusion of the splenic vein or superior mesenteric vein has a different causal spectrum and clinical presentation, and is hence removed from the defining criteria of EHPVO [4]. EHPVO is the most common cause of non-cirrhotic, presinusoidal, and prehepatic PH in children [4]. EHPVO represents also the most common disease on long-term care of PH, since these patients do not progress to end-stage liver disease and have no indication for liver transplantation. As a consequence, they represent the group of pediatric patients in whom there is the largest experience with long-term complications and care of PH [78, 133]. EHPVO is primarily a childhood disorder but can present at any age from few months to adulthood.

The etiology of EHPVO is not yet well defined, but various factors including umbilical vascular catheterization, sepsis, and an underlying hypercoagulable states (or thrombophilia) play a key role in the pathogenesis of the thrombus formation. Due to that a full hypercoagulability

panel, including genetic factors, has to be performed whenever the diagnosis is made [22]. Pathogenetic mechanisms which lead to PH are mainly related to the increased vascular resistance in the portal venous system due to thrombus formation. The formation of the portal cavernoma represents a tentative to bypass the thrombus and replace a physiological portal venous flow.

Studies on adult patients with EHPVO have been performed to assess the role of PH in producing changes in splanchnic and systemic circulation in the absence of liver dysfunction. They demonstrated an increase in the cardiac index and a decrease in the total peripheral resistance in patients with EHPVO compared to control patients, suggesting the presence of a hyperdynamic circulation also in patients with a normal liver function. Systemic and pulmonary hemodynamic changes have been evaluated in adults with EHPVO and compared with a group of controls represented by patients with compensated cirrhosis. The authors considered the measurements of cardiac index (by Fick's oxygen method), and systemic and pulmonary vascular resistance indices. Both patients with EHPVO and cirrhosis had similar values, confirming that patients with EHPVO have a hyperdynamic circulation similarly to cirrhotic compensated patients who have the same degree of PH [78, 134, 135]. These studies suggest a predominant role of PH per se in the genesis of systemic and pulmonary hemodynamic alterations [136].

Expanded plasma volume, development of porto-systemic venous collaterals, and increased venous return to the heart seem to be the main factors which cause and maintain hyperdynamic circulation in patients with EHPVO [133, 137–140].

There are no studies evaluating the presence of a hyperdynamic circulation in children with EHPVO. Radiological procedures to assess hemodynamic changes (i.e., HVPG, right atrial pressure, pulmonary arterial pressure, pulmonary wedge pressure, and mean arterial pressure) are considered too invasive and are not routinely performed in children except in selected cases [125].

Nevertheless, children with EHPVO usually do not show symptoms compatible with the hyperdynamic circulation such as warm extremities, cutaneous vascular spiders, wide pulse pressure, and capillary pulsations in the nail beds. Even major complications of the hyperdynamic circulation of cirrhosis (high cardiac output, HRS, SBP), described in animal models and adult patients, are not common in children with EHPVO. The symptoms at onset are mainly related to the complications of PH and may influence the outcome. In a multicenter Italian study on 187 children, the authors demonstrated that the diagnosis of PVT followed the detection of splenomegaly (40% of cases), gastrointestinal bleeding (36%), and hypersplenism (6%); conversely, the diagnosis was incidental in 18% of cases. Noteworthy, the authors showed that spleen size, variceal bleeding, and hypersplenism at presentation were predictors of surgery or radiological procedure ($p < 0.05$) [78].

Abdominal pain, ascites, or fever in the absence of portal cavernoma and porto-systemic collaterals are suspected for PVT with an acute presentation (pylephlebitis). On physical examination, the liver is normal or shrunken. Liver function tests are usually normal, at least in the early phases, whereas they can be deranged in the long term [141]; in fact, the increase of γ-glutamyl transpeptidase, total bilirubin, and bile salts in this setting should raise the suspicion of the development of portal hypertensive biliopathy (PHB) [142–144].

In clinical practice, EHPVO is considered a less severe form of PH. The patients may be asymptomatic for many years, and the mortality from bleeding appeared to be negligible in this group of patients [145, 146]. The diagnosis is based on Doppler ultrasound, CT scan, or nuclear magnetic resonance (NMR), which demonstrates portal vein obstruction, presence of intraluminal material, or portal vein cavernoma [72, 147].

Invasive procedures, such as transjugular retrograde or percutaneous transhepatic portal venography, should be undertaken when uncertainty persists. Liver biopsy is not essential for the diagnosis unless an underlying chronic liver disease is suspected, but, when performed, has shown a picture similar to what is described in NCPF. Echocardiography may rule out associated congenital heart disease, and look for HPS or PPH. Children with EHPVO are usually diagnosed years after the event. Anticoagulation therapy is not indicated outside of the acute phase, unless a hypercoagulable state has been documented [11].

Growth Retardation

Incidence and natural history of EHPVO in children is not well defined. The morbidity is mainly related to variceal bleeding, hypersplenism, and overall limitation of quality of life. The management of variceal bleeding in non-cirrhotic PH does not differ from what we described in cirrhotic patients. However, other complications, in both the short and the long term, need to be further elucidated. Growth retardation represents an important complication in this setting. Failure to thrive in children with EHPVO depends on duration of PH and declines further with age despite appropriate energy intake. Growth failure in EHPVO is multifactorial and is caused by chronic reduction of hepatic blood flow with concomitant decrease in hepatotropic factors, malabsorptive state caused by portal hypertensive enteropathy, early satiety caused by massive splenomegaly, a state of growth hormone (GH) resistance shown by high levels of GH, and low levels of insulin-like growth factor (IGF)-1 and

IGF-binding protein-3, and lastly caused by anemia and thrombocytopenia. Restoration of portal blood flow to the liver that follows a successful meso-portal bypass (MPB) results in improved growth in these patients [147–150] (Fig. 71.5).

Portal Hypertensive Biliopathy

Patients with EHPVO occurring in infancy almost invariably develop radiological evidence of PHB as young adults; nevertheless, only 20–30% develop clinical signs of cholestasis [151]. The term PHB was coined to describe a disorder characterized by anatomical and functional abnormalities of the intrahepatic, extrahepatic, and pancreatic ducts occurring most commonly in patients with non-cirrhotic PH [142]. The definition needs exclusion of other biliary diseases. Abnormalities of the biliary tree include intrahepatic biliary radicle dilatation, caliber irregularities, displacements, ectasias, strictures, and common bile duct stones [144]. Pathogenesis is related to two mechanisms: compression of the common bile duct by dilated collaterals and neovascularization secondary to long-standing PVT and bile duct ischemia caused by prolonged compression by collaterals, thrombosis of smaller veins draining the duct, or excessive deposition of connective tissue forming a tumorlike cavernoma [143, 147]. Frequency of PHB is highest in patients with EHPVO (80–100%) in contrast with cirrhosis (0–33%) and IPH (9–40%). Most (62–95%) patients are asymptomatic [147–150].When symptomatic, PHB presents with jaundice, biliary colic, abdominal pain, and recurrent cholangitis. Magnetic resonance cholangiopancreatography (MRCP) is the first-choice tool to diagnose PHB in children [152]. The decision to treat biliary obstruction in these patients depends on the presence of symptoms. In asymptomatic patients no intervention is recommended. In symptomatic children, biliary stenting (by endoscopic retrograde cholangiopancreatography, ERCP, or percutaneous transhepatic cholangiography, PTC) may temporarily improve the symptoms restoring a normal bile flow. Natural history of biliopathy is poorly defined, but a slow progression is well known, with development of symptoms over a decade. Overall 4–10% of patients develop complications like choledocholithiasis, cholangitis, and secondary biliary cirrhosis [147–150]. Therefore, some patients may require shunt or bypass surgery to decompress the biliary varices and resolve the obstruction [151].

Fig. 71.5 Twins who were born preterm and had cannulation of the umbilical vein. One developed portal vein thrombosis and shows evident growth retardation

Minimal Hepatic Encephalopathy

HE is a brain dysfunction caused by liver insufficiency and/or porto-systemic shunting; it manifests as a wide spectrum of neurological/psychiatric abnormalities ranging from subclinical alterations to coma. The subclinical manifestation of HE, which is called minimal HE (MHE), is detectable by the alteration of at least two specific psychometric tests or electrophysiological techniques [114]. Nowadays, the term covert HE is also used to refer to all the spectrum of manifestations of HE that do not produce disorientation in time or space [153].

HE can occur not only in patients with liver cirrhosis but also in those with non-cirrhotic PH and porto-systemic shunting [154, 155]. MHE has been reported in about one third of children with EHPVO and normal liver function [156, 157]. The diagnosis is made by psychometric tests, critical flicker frequency, and MR spectroscopy [158]. Hyperammonemia seems to play a key role in the pathogenesis of this complication [159].

According to this research, MHE would compromise attention, processing speed, and psychomotor performance, in some cases affecting the academic performance of the patients. MHE seems solved by restoring blood flow to the liver by the meso-portal bypass (MPB), while surgical porto-systemic shunts may eventually worsen it [114, 156].

Management of EHPVO

Management of children with EHPVO is primarily focused on treatment of PH complications. However, the use of medical therapy, endoscopic procedures, and surgery is still questionable because there are no evidence on efficacy of NSBBs, endoscopic variceal obliteration (EVO), and different types of surgical operations [72]. In clinical practice, EHPVO is managed according to what is proposed for cirrhotic children with PH. As far as surgery is concerned, special attention should be paid to the possibility of curing these patients by a successful MPB. MPB represents a physiologic repair of

EHPVO, restoring the normal hepatic physiology, and therefore it should always be considered in this setting [160]. However, there is no wide agreement on feasibility, indications, timing, and success of this procedure [161, 162]. Due to the absence of standardized guidelines, the management of EHPVO needs to be individualized depending on the age of presentation, site and nature of obstruction, and clinical manifestations. In a retrospective study, we reviewed 65 children with EHPVO (median age at diagnosis 3.5 years) and proposed a stepwise approach to manage such a cohort of patients. After retrograde portogram, MPB resulted to be feasible only in 44% of the cases. Children were treated with endoscopic procedures and NSBB as first-line therapy. Those who had varices not well controlled by medical/endoscopic treatment underwent MPB in 13 (38.2%), a proximal splenorenal shunt in 13 (38.2%), a meso-caval shunt in 3 (8.8%), a TIPS in 2 (5.9%), a distal splenorenal shunt in 2 (5.9%), and a LTX because of HPS in 1 (3%). Such a stepwise approach, consisting of medical, endoscopic, and surgical options, provided excellent survival and bleeding control in more than 90% of the patients [2]. In a large study on children with PVT (n = 187), after a median follow-up of 11.2 years, 124 patients (66%) were treated conservatively, and 63 (34%) had undergone surgical or radiological procedures including meso-portal bypass in 30 (48%), distal splenorenal shunt in 16 (25%), proximal splenorenal shunt in 7 (11%), meso-caval shunt in 4 (6%), isolated splenectomy in 2 (3%), TIPS in 2 (3%), porto-caval shunt in 1 (2%), and LTX in 1, because of development of hepatopulmonary syndrome. At last follow-up the surgical or radiological shunt (total n = 60) was patent in 54 patients (90%) and obstructed in 6 (10%, 4 with proximal splenorenal shunt, 1 with meso-portal bypass, and 1 with meso-caval shunt) [78].

A lively debate is ongoing as to whether MPB should be considered as a preemptive technique or as a second-line option after failure of medical and endoscopic management [162, 163]. Although MPB may turn up not to be feasible in children who developed EHPVO following neonatal umbilical catheterization, it seems reasonable to consider restoring the normal liver flow in the early phase of the disease, whenever possible [2, 164].

Diagnosis of Portal Hypertension

The diagnostic workup in patients with PH includes actions aiming at diagnosing the underlying liver disease, quantifying the degree and severity of PH, and identifying the presence of clinical complications. In the clinical history, it is important to collect information on prematurity, neonatal jaundice, umbilical catheterization, and presence of signs or symptoms highly suspicious for PH (e.g., history of unexplained splenomegaly) (Table 71.3).

Physical examination is directed to assess liver size and consistency, splenomegaly, abdominal venous pattering (site and direction of venous flow), ascites, skin signs of chronic liver disease (e.g., spider nevi, telangiectasias, palmar erythema), bruises, and edema.

Laboratory tests should include liver function, blood cell and platelet count, and clotting.

A variety of radiological and endoscopic procedures are routinely utilized in children to diagnose PH. However, the majority of them have been well studied in adults but not in the pediatric population. Such procedures include abdominal Doppler ultrasound, upper GI endoscopy, CT scan of the abdomen, invasive measurements of portal venous pressure, and liver biopsy.

Doppler Ultrasound

Doppler ultrasound is a noninvasive and inexpensive technique that is widely used in children to study liver vessels and parenchyma. Although operator dependent and related to the experience and skill of the radiologist, Doppler ultrasound is a valuable tool to screen patients with suspected PH both at the time of the diagnosis and during the follow-up. In a few minutes, this test can provide information on liver size and texture, patency of portal and hepatic veins, hepatic artery patency and flow pattern (including the resistance index), porto-systemic shunting, ascites, splenomegaly, and associated intra-abdominal abnormalities [165].

The liver is usually enlarged in the hepatic (e.g., biliary atresia, ciliopathies, genetic cholestasis) and posthepatic forms of PH (e.g., Budd–Chiari syndrome), whereas it is of normal size in prehepatic PH. The echogenicity of the parenchyma may be increased in cirrhosis and in some diseases in which steatosis is a histological feature (i.e., Wilson's disease and α1-antitrypsin deficiency) [166]. Gross abnormalities of the bile ducts, such as biliary dilatation, the presence of gallstones, or other morphological abnormalities, can be easily visualized by ultrasound, whereas small irregularities require more powerful imaging studies to be detected.

In patients with prehepatic PH, it is crucial to detect the presence of a portal cavernoma and rule out a dilatation of the biliary tree possibly due to PHB. The splenic size is easily measured and compared to normal values for age, although it does not correlate strictly with the severity of PH [165]. In children with liver disease, it is important to evaluate also the renal parenchyma to exclude the presence of renal cysts that can accompany several genetic liver disorders and provide a further hint to the diagnosis [166].

Bidimensional ultrasonography can easily detect and confirm the presence of ascites suspected clinically, and the color Doppler technique provides information on blood flow in the portal venous system, the hepatic artery, and the

hepatic veins, where it is possible to calculate the flow velocity, although it is not possible to estimate pressures [167, 168]. When PH worsens, the portal blood flow may become hepatofugal toward the left gastric, paraduodenal, or paraumbilical veins. Reversal of flow in the SMV or SV may be suggestive of spontaneous mesentericocaval or splenorenal shunts, respectively [169].

The hepatic veins are straight, anechoic, tubular structures that converge toward the IVC approximately 1 cm below its confluence with the right atrium. The normal hepatic vein waveform is triphasic as a result of transmitted cardiac activity [170].

Varices are formed in the lower esophagus by portosystemic shunting via the left gastric vein through the lesser omentum (Fig. 71.1). As a consequence, the lesser omentum gets thickened in PH [171, 172]. Patriquin H et al. measured the lesser omental thickness in 150 children without systemic, liver, or renal disease. They suggested that, in the absence of obesity or lymphadenopathy, a lesser omentum measuring more than 1.7 times the aortic diameter should raise the possibility of PH [173].

Although the "gold-standard" method for liver fibrosis assessment is liver biopsy, in the past years, noninvasive methods have increasingly been used in adult hepatology. The best validated tool is transient elastography (TE) [174, 175]. Data on its use in children are still scarce, and the influence of technical aspects such as probe choice and site of measurement on results is not clear. In one study TE was performed in 527 children (229 girls, ages 0.1–17.8 (median 6.0) years, including 400 healthy controls). The feasibility rate was 90%, but it decreased to 83% in children younger than 24 months even in ideal conditions. General anesthesia significantly increased liver stiffness in healthy children. The authors concluded that in one study, TE is feasible even in extremely young children, but confounding influences on test results such as probe choice, sedation, or food intake need to be taken into account when interpreting the results [176].

Endoscopy

Unlike adults, in the pediatric population, there are few reports on the prevalence of varices in children with PH, and it is therefore difficult to predict how many children would benefit from endoscopic screening [177]. In children, the endoscopic procedures to diagnose and treat EV are routinely performed under general anesthesia.

There is no recommendation to routinely undertake tests to screen for the presence of varices in children with PH. Despite that, many pediatric hepatologists prefer their patients to undergo endoscopic surveillance to best define and prevent the risk of bleeding from varix rupture. In fact, when pediatric hepatologists were asked if they would offer screening endoscopy for varices to a child with biliary atresia and evidence of PH, most of them answered they would, both in Europe and in North America [3, 73, 160].

Data on diagnosis and grading of EV in children are scant. The scoring systems adopted in adults have not been validated in children, but such information is mandatory to determine the effectiveness of prophylaxis of variceal bleeding by either NSBBs or endoscopic treatment (Fig. 71.3). Studies on the interobserver agreement on pediatric varices grading suggest that accordance in the recognition of large varices is satisfactory [178].

Another major issue is how to grade varices in this setting. Varices have been defined into three grades according to the size, and red marks have been shown to predict bleeding. Recently, the classification has been simplified, and the proposed description of small or large varices, with or without red marks, appears to be more practical [76]. Large varices, varices of any size but with red marks, and gastric varices are likely at higher risk of bleeding in the short term, but again this has not been proven in children so far [179].

Endoscopy in children with PH has been indicated only for the treatment of acute bleeding and in the secondary prophylaxis of further bleeding episodes. The usefulness of diagnostic endoscopy and primary prophylaxis of bleeding by endoscopic variceal obliteration is still unproven, but may become common practice to prevent the shocking experience and the morbidity of a first bleed in a child with clinical evidence of advanced PH [72, 180].

Measurement of Hepatic Venous Pressure Gradient

PH is defined by an increased pressure in the portal venous system. Such an increased venous pressure may be detected by a direct measurement of the pressure into the portal vein or by the measurement of a portal pressure gradient (PPG) resulting from the difference, in pressure, between the portal vein and the IVC. Direct measurements of portal pressure can be performed through transhepatic or transvenous catheterization of the portal vein, but, since they have high risk of major complications (e.g., intraperitoneal bleeding), these tools are rarely used, apart from those cases in which WHVP is unreliable, such as presinusoidal PH [181].

HVPG measures the PPG as the difference between "wedged" hepatic vein pressure (WHVP) and "free" hepatic vein pressure (FHVP). The WHVP is measured by occluding the hepatic vein by inflating a balloon at the tip of the catheter. The injection of 5 ml of contrast dye into the vein with the balloon inflated can confirm an adequate occlusion of the hepatic vein. The WHVP reflects the portal vein pressure basing on the concept that when the blood flow in a hepatic vein is blocked by a "wedged" catheter, the static column of blood

transmits the pressure from the preceding vascular territory, in this case, the hepatic sinusoids. As in cirrhosis the intersinusoidal communications are lost due to fibrosis, septa, and nodule formation, the sinusoidal pressure equilibrates with portal pressure. Thus, the WHVP correlates closely with portal vein pressure, but, in fact, it is a measurement of the hepatic sinusoidal pressure and not of portal pressure itself [35].

The difference between WHPV and FHPV provides HVPG values (HVPG = WHVP − FHVP).

Normal HVPG ranges from 1 to 5 mmHg in adults. Subclinical PH is defined when HVPG ranges from 6 to 10 mmHg, whereas complications of PH are expected when HVPG is greater than 10 mmHg. An HVPG greater than 12 mmHg correlates with variceal bleeding, rebleeding, and increased mortality [181].

HVPG values allow to classify different forms of PH. Presinusoidal PH is characterized by normal or slightly increased HVPG values, with normal or slightly increased WHVP and normal FHVP. Sinusoidal PH is found in most chronic liver diseases and is characterized by an increase in WHVP with normal FHVP, resulting in high HVPG (cirrhosis is the most common cause). In postsinusoidal PH, HVPG is normal and both WHVP and FHVP are increased, such as in the Budd–Chiari syndrome (Table 71.2).

The HVPG is considered the gold-standard technique to measure portal venous pressure and, in cirrhotic adults, is widely utilized to quantify the severity of PH, predict the outcome, and guide the therapeutic decisions [4, 182, 183].

In children, the diagnosis of PH is essentially based on clinical evidence of PH complications (i.e., splenomegaly, upper varices, ascites) in a setting of an underlying liver disease. Unfortunately, so far the measurement of HVPG in children has been considered an invasive procedure that has to be performed only in limited cases. Due to that, we have only few published data on HVPG measurements in the pediatric setting [184]. Wolfsson reported on 49 children, with acute and chronic liver disease, who underwent 52 HVPG measurements. The procedure resulted feasible in all patients and no complications were documented. HVPG values ranged between 0 and 28 mmHg, and they were greater than 6 mmHg in 30 patients. The authors concluded that, despite the small sample size, HVPG measurements were feasible and safe in their cohort of patients [185]. Further studies on large cohorts of pediatric patients are necessary to obtain strong evidences on the utility of the HVPG measurements in the management of children with PH.

Other Investigations

CT scanning with intravenous contrast and MR angiography may be used to study children with PH. These investigations provide information on focal liver lesions, portal vein and hepatic vein patency, presence of collateral circulation, and arteriovenous shunts. CT has a sensitivity of 85% in the detection of EV compared to endoscopy, but has the advantage of demonstrating splenorenal, gastrorenal, peripancreatic, pericholecystic, retroperitoneal, and omental collaterals, as well as spontaneous large porto-systemic shunts [186]. In a study performed on adult patients, MR angiography proved more reliable than Doppler ultrasound for evaluating the portal venous system in patients with PH caused by cirrhosis [187].

Management of Portal Hypertension

Prophylaxis of Bleeding

Currently there are no data supporting the role of any type of prophylaxis to prevent variceal bleeding in children [3]; nevertheless, many clinicians would consider a cirrhotic child with large varices at risk of mortality from the first bleed, and therefore a definite candidate for primary prophylaxis [73]. Conversely, a reasonable, and somehow evidence-based, consensus on indication to perform endoscopic secondary prophylaxis (prevention of rebleeding) in cirrhotic children appears to be wide [72].

Nonselective β-Blockers

The rationale of NSBBs in PH stands on its ability to decrease the portal flow by reduction of cardiac output (via β1-receptor antagonism) and splanchnic vasodilatation (via β2-receptor antagonism) [188]. Studies in adults have shown that a dose reducing the heart rate by 25% (or the HVPG by 20%) does decrease the bleeding rate in cirrhosis [183]. There are no randomized trials assessing the efficacy of propranolol as prophylaxis of variceal bleeding in children, and the few cohort studies carried out did not include the measurement of HVPG before and after treatment start [6, 189, 190]. Moreover, these studies showed that in children, the evaluation of heart rate at rest is problematic, and the range of drug dosage required to reduce it by 25% is very wide, making achievement of adequate NSBB dosage impractical and time-consuming. Whether pediatric patients with presinusoidal PH, having no classical features of the hyperdynamic circulation, may benefit from treatment with NSBBs has yet to be demonstrated [140].

A further challenge in carrying out trials with NSBBs in this setting is that propranolol is not licensed for use in children [191]. The relative difficulty to dose this drug along with its side effects on physical performance in young and growing subjects may lead to downgrade its benefits in the long term, and reconsider the use of this drug in the prophylaxis of variceal bleeding in children.

Endoscopy for Screening and Management of Esophageal Varices

There are few reports on the prevalence of varices in children with PH, and it is therefore difficult to predict how many would benefit from endoscopic screening. Besides, the uncertainty regarding the impact of any prophylaxis in this setting makes endoscopic screening questionable.

Despite this, in some reports the mortality of cirrhotic children at the time of first bleeding episode was as high as 5–15%, and morbidity was shown to reach 57% of all subjects. This may support the decision to do screening endoscopy in all children with advanced liver disease and clinical signs of PH [80, 180].

Unlike the past decades when endoscopic obliteration of varices was done using sclerosing agents (such as ethanolamine or polidocanol) injected inside or around the varix, currently endoscopic variceal ligation (EVL) has become more popular and has been shown to be superior to sclerotherapy as far as efficacy, safety, and degree of standardization are concerned, in both adults and children [192–193]. Nevertheless, in small children, in whom the banding devices available on the market cannot be used with small pediatric endoscopes, sclerotherapy remains the only feasible treatment option to manage large varices [193].

Endoscopic treatment is usually safe and well tolerated by children, but often it has to be continued for an undefined period of time. Authors reported the results from a single-center study of primary and secondary prophylaxis of bleeding in 66 children with biliary atresia complicated by PH and high-risk varices. Thirty-six children (mean age, 22 months) underwent primary prophylaxis, and 33 (mean age 24 months), who presented with gastrointestinal bleeding, received endoscopic treatment to prevent a relapse of bleeding (secondary prophylaxis). In the primary prophylaxis group, a mean number of 4.2 sessions were needed to eradicate varices; no bleeding from gastroesophageal varices was observed after eradication, and varices reappeared in 37% of cases. In the secondary prophylaxis group, a mean number of 4.6 sessions was needed to eradicate varices, and varices reappeared in 45% of cases. These results showed that endoscopic therapy appeared to be well tolerated and greatly reduced the risk of variceal bleeding. However, the recurrence of varices suggested that the continued endoscopic surveillance was needed [194]. A real challenge in this setting is the presence of large gastric varices; there are no published data on experience of management of gastric varices in children, and probably most centers would treat this scenario according to the experience in adults. Large gastric varices are a threat because they are difficult to obliterate prophylactically, and even more so if actively bleeding; in this situation, balloon tamponade is often ineffective, and the only option is to perform sclerotherapy with tissue glue (such as *N*-butyl-cyanoacrylate). In general, a child with large gastric varices should be considered for TIPS, shunt surgery, or liver transplantation, based on the degree of liver disease.

Management of Acute Variceal Bleeding

The main goal of the management of a child with acute esophageal bleeding is well-balanced blood volume restitution. It is therefore mandatory to monitor vital signs, obtain venous access to perform blood tests (full blood count, international normalized ratio, liver function and electrolytes, C-reactive protein, and a blood crossmatch), and start blood volume correction [195]. Packed red blood cells (PRBC) should be provided with the aim to maintain the hemoglobin >7 g/dl, carefully avoiding a rebound overload of fluids that favor the increase of portal pressure and rebleeding [196]. In the presence of coagulopathy, it might be wise to support the patient with plasma, also in view of the fact that esophageal bleeding implies loss of whole blood that, if large, will not be efficiently replaced by PRC. Children with upper GI bleeding may benefit from nasogastric tube placement, with the primary goal being to monitor persistence of active bleeding. Vasoactive drugs, such as octreotide, are effective in stopping bleeding from varices and should be started immediately to bridge the child to endoscopy, and continued thereafter for a total of 4–5 days [197].

In adults, it has been proven that infectious complications commonly follow an episode of variceal bleeding in cirrhotic patients [198]. Although in children there is no such evidence, it is recommended to monitor them for any sign of infection and, if present, to start antibiotic treatment promptly, especially in cirrhotic children with advanced disease.

After the initial step, the child should be managed according to hemodynamic stability and the control of bleeding. If unstable, the child should be managed in an intensive care setting, possibly with a central venous catheter providing information on circulating blood volume and preload (Fig. 71.6). Usually, bleeding stops spontaneously after the ruptured varix empties. After cessation, it is usually acceptable to schedule an elective endoscopy in the following 24–72 h because rebleeding is uncommon during this time frame. If bleeding does not stop despite appropriate fluid replacement and correction of coagulopathy, the child may require urgent endoscopy and, rarely, the placement of a Sengstaken balloon as a bridge to TIPS or urgent shunt surgery (Fig. 71.7). Endoscopic sclerotherapy around the vessel may be the only option to treat an acutely bleeding varix that is underfilled, and therefore difficult to be strangulated by a rubber band placed by endoscopic variceal ligation devices.

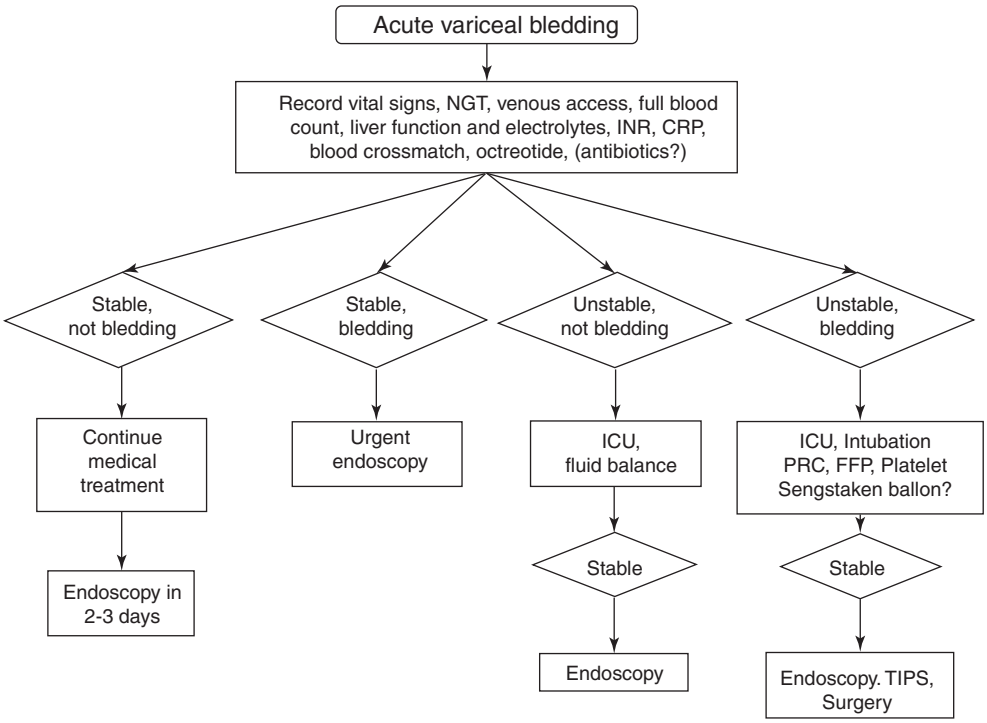

Fig. 71.6 Proposed algorithm for the management of acute variceal bleeding. NGT nasogastric tube, INR international normalized ratio, CRP C-reactive protein, ICU intensive care unit, PRC packed red cells, FFP fresh frozen plasma, TIPS transjugular intrahepatic porto-systemic shunt. (Reprinted from Ref. [73], with permission from Elsevier

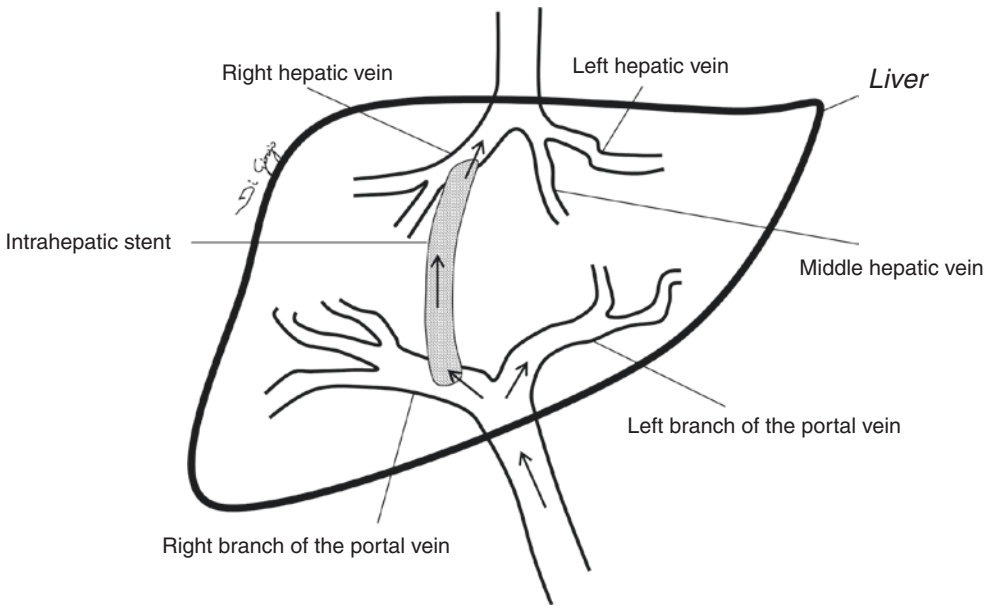

Fig. 71.7 Portal circulation after the placement of transjugular intrahepatic porto-systemic shunt

Surgical Procedures

When medical and endoscopic treatment of bleeding varices fails, the only option is to consider decompression of the portal system by a shunt or a bypass [199]. Children with EHPVO can be managed effectively by MPB (Fig. 71.8) [23]; however, in our experience of children who had an umbilical venous catheter placed at birth, only about half had a patent Rex recessus at retrograde portogram [2]. If the MPB is not feasible, these patients can usually be treated by other forms of shunt surgery [200]. One recently suggested approach is to perform the MPB preemptively, regardless of complications of PH, in view of its beneficial effects on growth and neurocognitive outcome [162, 164, 165]. Other patients with presinusoidal PH, but not amenable to MPB, can be managed by TIPS or by shunt surgery [201].

Nonphysiological porto-systemic shunts are still the commonest surgeries performed for EHPVO worldwide. There is a vast literature on feasibility, patency, and long-term outcomes of these shunt surgeries; however, the long-term

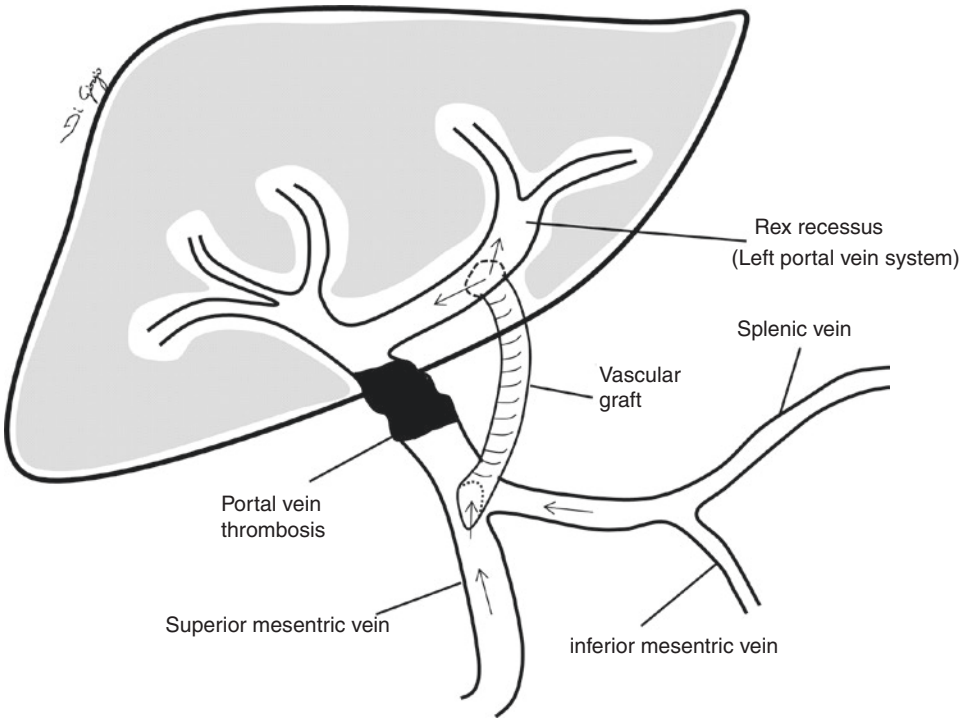

Fig. 71.8 Portal circulation after the operation of meso-portal bypass that reestablishes the hepatopetal flow to the liver

patency has been questioned. While nonselective shunts, like proximal splenorenal shunt, decompress whole system taking care of PHB as well, the distal splenorenal shunt treats the left-sided PH. Furthermore, in younger children there are issues related to technical feasibility and shunt thrombosis, but these have been largely overcome with improvement in surgical expertise [165]. Cirrhotic children with PH usually have a rapidly progressive biliary type of cirrhosis (such as biliary atresia, intrahepatic cholestasis, and Alagille syndrome), are young, and have a short transplant-free survival. In our institution, the median age at transplantation is 1.4 years. Therefore, shunt surgery or TIPS is rarely indicated in this cohort of patients in whom PH is usually accompanied by liver decompensation and is an indication for LT. However, cirrhotic children with a compensated longstanding noncholestatic liver disease complicated by severe PH may be considered for TIPS.

Transjugular Intrahepatic Porto-systemic Shunt

TIPS is a well-established tool to manage severe complications of PH in adults, but its experience in children is limited because it is thought to be associated with high risk of shunt dysfunction, and to offer short-term benefits. As a consequence, in pediatrics TIPS is regarded mainly as a bridge to liver transplantation in children with cirrhotic PH. At our center we performed a study to assess the technical feasibility, clinical efficacy, and shunt patency in a cohort of children with both cirrhotic and NCPH. Twenty-nine children (cirrhotic-PH = 11, NCPH = 18, mean age 10.3 years, ±4.3) underwent TIPS procedure for recurrent variceal bleeding ($n = 18$) and refractory ascites ($n = 11$). The intrahepatic shunt was successfully placed in 27/28 patients, the porto-systemic gradient reduced to δ12 mmHg, and complications of PH were solved in 26/27 cases making the technical success rate, the hemodynamic success rate, and the clinical success rate of 96%. Primary patency rates at 6 months and at 1, 2, and 4 years were 91%, 83%, 60%, and 46%, respectively; remarkably, primary assisted and secondary patency rate were 100% without major differences between the two groups of patients. No patient developed HE. The results from our study clearly demonstrated that TIPS appears to have a high mid-term patency rate. Its high clinical success rate, along with a minimally invasive approach, suggests that TIPS should not be regarded only as a bridge to transplantation but also as an effective and less invasive alternative to surgical vascular shunts in children with NCPH [88]. Therefore, TIPS appears to be feasible and effective in children as it is in adults and should become part of the armamentarium used to manage PH complications in pediatric patients.

A Protocol for Screening, Prophylaxis, and Treatment of Esophageal Varices

The need for large sample sizes, the difficulties in recruiting patients into multicenter studies, and the lack of official approval and knowledge on drug dosing make it quite

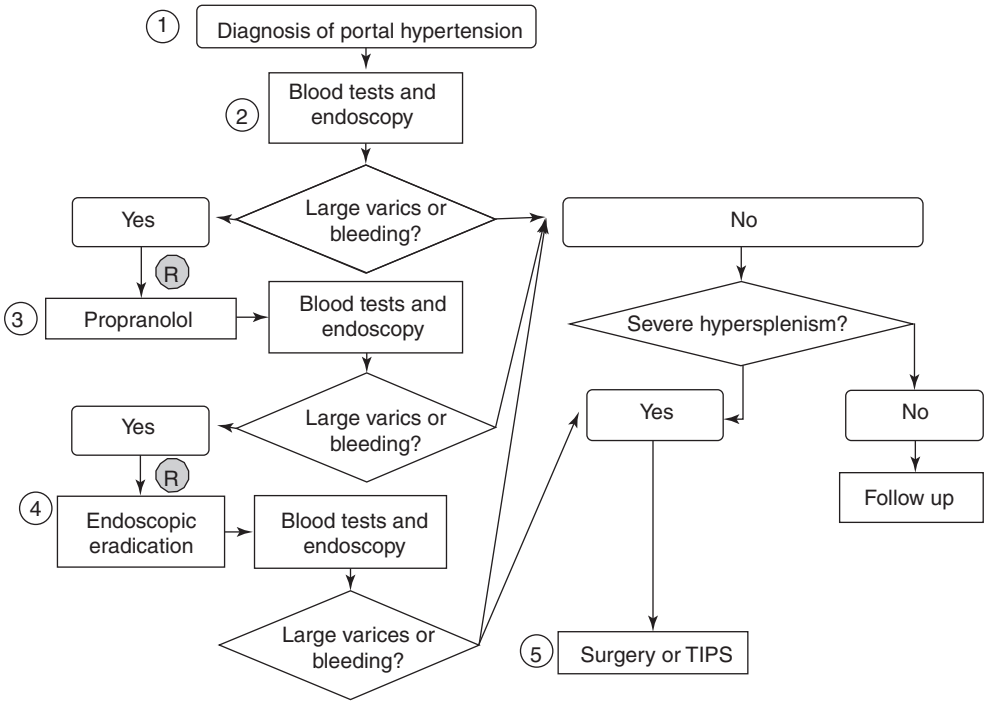

Fig. 71.9 Proposed algorithm for the approach to the child with portal hypertension. TIPS transjugular intrahepatic porto-systemic shunt. The circled "R" represents a step for possible randomization in a clinical trial. (Reprinted from Ref. [73], with permission from Elsevier)

unlikely that there will be robust data on the use of NSBBs in children with PH in the coming years. The same applies to endoscopy because there is no single-center able to recruit enough patients to answer questions regarding screening and primary prophylaxis of varices, and multicenter trials require diagnosis and treatment standardization. Besides, such studies can probably only be carried out in non-cirrhotic children having sufficient follow-up time to test the given hypothesis. Alternatively, many centers are already using these tools empirically in both cirrhotic and non-cirrhotic children, with uncertain and inconsistently measurable results. Is it then possible to gather more information on the utility of NSBBs and endoscopic treatment of varices in children? A proper trial on this matter should be randomized and have variceal bleeding as the primary end point; however, many clinicians and families would consider permitting a GI bleed as unacceptable to test the hypothesis of effectiveness of NSBBs or endoscopic treatment. One possibility to overcome this could come from considering the development of large varices as the end point, because children with large varices or red marks have failed treatment and will eventually bleed. At least two studies have shown that most children with cirrhosis or PVT and grade 2–3 varices will bleed within a few years of follow-up [202, 203]. Therefore, it is possible to hypothesize a randomized, nonblinded multicenter trial of development of large varices in children and their response to treatment. Because of the large sample size needed, such study would first require a solid proof that there is sufficient agreement among endoscopists to recognize large varices in the different pediatric centers involved in the trial [179].

Figure 71.9 illustrates an algorithm of a stepwise approach to manage EV, which considers the formation of large varices as the end point and could offer the chance to test the hypothesis that NSBBs and endoscopy can improve the outcome of children with PH, avoiding the risk of not offering the best of practice currently available. Nevertheless, an extraordinary effort will be required to produce such evidence on the best management of PH in children.

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Liver Tumors in Children

72

Mohamed Rela, Ashwin Rammohan, and Mettu Srinivas Reddy

Introduction

Liver tumors are uncommon in the pediatric age group and constitute 1–2% of all solid tumors in children. About 60% of all liver tumors in children are malignant [1]. Hepatoblastoma (HB) is by far the most common constituting over 50–60% of all liver tumors in this age group. Hepatocellular carcinoma (HCC), undifferentiated embryonal carcinoma, and biliary rhabdomyosarcoma are other malignant liver tumors in children. Benign tumors include hemangiomas (HMGs), mesenchymal hamartoma, and focal nodular hyperplasia (FNH). Secondary tumors to the liver can spread from a host of primary tumors including lymphomas, Wilms' tumor, neuroblastoma, osteosarcoma, etc. (Table 72.1). Several congenital and environmental risk factors have been reported to increase the predilection for liver tumors (Table 72.2).

There is a striking age-related variation in the frequency of different tumor types (Table 72.3). Over 90% of liver tumors in children below 5 years are HB, while 87% of tumors in the 15–19-year age group are HCC. A gradual increase in the incidence of liver tumors in children over the past 3–4 decades has been reported. This is particularly evident in the case of HB where the incidence has increased from 0.6 to 1.2 per million population between 1973 and 1977 and 1993 and 1997. On the contrary, incidence of HCC has decreased from 0.45 to 0.29 per million population during the same period [2].

Tumor Markers in Childhood Liver Tumors

Alpha-fetoprotein (AFP) is the most recognized tumor marker in liver tumors. AFP is a glycoprotein similar in physical and chemical characteristics to albumin. It is

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Table 72.1 Types of liver tumors in children

Benign	Hemangioma: focal, multiple, diffuse
	Mesenchymal hamartoma
	Hepatic adenoma
	Focal nodular hyperplasia
	Inflammatory myofibroblastic tumor (may be locally invasive)
Malignant	
Primary	Hepatoblastoma
	Hepatocellular carcinoma, fibrolamellar HCC
	Transitional tumors
	Embryonal sarcoma
	Biliary rhabdomyosarcoma
	Calcifying nested stromal–epithelial tumor
	Angiosarcoma
Secondary	Lymphomas, leukemia, Wilms' tumor, neuroblastoma, osteosarcoma, colon cancer

Table 72.2 Risk factors and premalignant conditions for childhood liver tumors

Tumor	Risk factors	Premalignant lesions
Hepatoblastoma	Beckwith–Wiedemann syndrome, familial adenomatous polyposis, Li–Fraumeni syndrome, trisomy 18, preterm birth, and very low birth weight	–
Hepatocellular carcinoma	Glycogen storage disease, hereditary tyrosinemia, Alagille syndrome, biliary atresia, PFIC, ataxia–telangiectasia, hepatitis B infection, hepatitis C infection	Hepatic adenoma
Embryonal sarcoma		Mesenchymal hamartoma
Hepatic angiosarcoma		Multiple/diffuse hepatic hemangioma

PFIC progressive familial intrahepatic cholestasis

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Table 72.3 Age-wise distribution of childhood liver tumors

Age group	Benign tumors	Malignant tumors
Neonatal period	Hemangioma, mesenchymal hamartoma	Hepatoblastoma
0–5 years	–	Hepatoblastoma, biliary rhabdomyosarcoma
5–15 years	–	Hepatocellular carcinoma, embryonal sarcoma
>15 years	Hepatic adenoma	Fibrolamellar carcinoma

secreted by the fetal liver and yolk sac until 13 weeks' gestation and then primarily by the fetal liver [3]. AFP levels at birth are very high with Bader et al. reporting a median level of over 40,000 ng/ml in cord blood samples. These levels rapidly drop during the first year of life at a rate primarily dictated by the half-life of AFP of 5–6 days [4]. AFP levels at birth in the preterm babies are higher than full-term babies. Similarly, a decrease in at-birth AFP for every week of prolonged gestation has been reported. The high levels of AFP in the infant should be kept in mind by the clinician when AFP levels are used for the diagnosis and monitoring of pediatric liver tumors.

Several tumors are associated with elevated AFP. HB is the commonest cause in infants though HCCs and germ cell tumors are also associated with elevated AFP. Nonneoplastic conditions, such as tyrosinemia and neonatal hepatitis, can also cause elevated AFP levels.

Malignant Tumors

Hepatoblastoma

HB is the most common liver tumor in children. It is almost always seen in the first 4 years of life with median age at diagnosis of 18 months. It can present at birth and has been diagnosed in the intrauterine period on antenatal scans. Prematurity and very small birth weight have been identified as risk factors. HB developing in these children is also reported to have a worse prognosis [5]. Data from the USA has shown a gradual increase in the incidence of HB over the last 40 years from 0.6 to 2.3 per million children [6].

HB is a tumor of immature hepatocyte progenitor cells. It is an embryonal tumor, which recapitulates various stages of liver development. Histologically, these tumors are heterogeneous and comprise combinations of epithelial, mesenchymal, and occasionally teratoid components in varying proportions (Table 72.4, Fig. 72.1). Majority of tumors have a primarily epithelial component containing hepatoblasts at varying stages of differentiation. The histological type has an impact on behavior with well-differentiated fetal epithelial type having the best prognosis. The small-cell undifferentiated type of HB has the worst prognosis with very poor survival. Mixed tumors contain both epithelial and mesenchymal components, are more resistant to chemotherapy, and have a worse prognosis. Post-chemotherapy residual tumors and metastatic tumors may demonstrate a pleomorphic pattern with pleomorphic nuclei having coarse chromatin and prominent nucleoli. This pattern may resemble HCC.

Table 72.4 Histological types of hepatoblastoma

Histological type	Description
Epithelial	Primarily containing immature hepatocytes
Fetal	Commonest variant of epithelial HB. Composed of polygonal cells resembling fetal hepatocytes arranged in one- to two-cell thick cords, trabeculae, or sheets
Embryonal, well differentiated	Tumor resembles the liver at 6–8 weeks of gestation. It demonstrates organized tubular or acinar formation. Hematopoietic elements are commonly admixed with epithelial component
Cholangioblastic	Tumor cells differentiate as cholangiocytes and form small ducts. This component expresses cholangiocyte lineage markers (cytokeratins 7 and 19)
Macrotrabecular	Cells arranged in thick trabecular pattern (>5 cells thick)
Small cell undifferentiated	Sheets of small cells with large hyperchromatic nuclei similar to neuroblastoma
Mixed epithelial–mesenchymal type	Mixture of epithelial and mesenchymal cell types
Teratoid	Contains heterologous components such as stratified squamous epithelium, mucus-producing cells, neuroectodermal derivatives
Non-teratoid	Contains stromal derivatives including spindle fibroblastic cells, osteoid, skeletal muscle, and cartilage

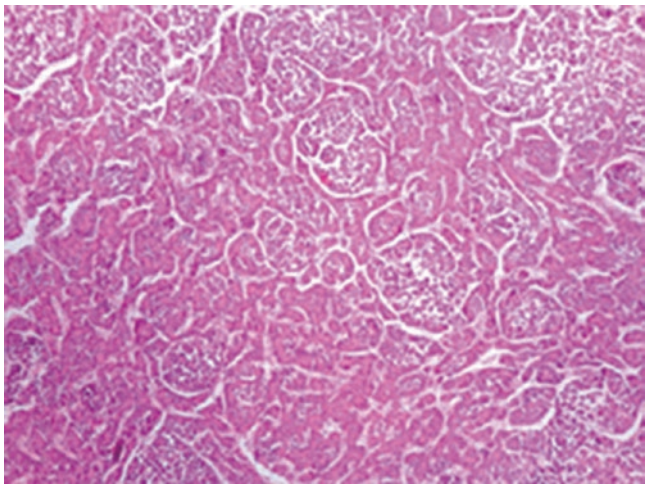

Fig. 72.1 Hepatoblastoma, epithelial type with fetal and embryonal epithelium

Diagnosis

The usual presentation is with an abdominal lump identified by the parent or the clinician. Pain, failure to thrive, and jaundice are uncommon modes of presentation. Investigations reveal an elevated AFP level in over 90% of cases. The AFP levels are extremely high (in the order of 10^5 ng/ml) and are usually a log higher than those seen with HCC. AFP level is a prognostic marker in HB with both very high levels and low levels (<100 ng/ml) predicting poor biology [7]. Thrombocytosis is a recognized laboratory finding in HB and is probably related to production of thrombopoietic cytokines including thrombopoietin in the tumor tissues. Contrast-enhanced computed tomography (CT) of the abdomen and a CT of the chest are necessary to confirm the diagnosis, stage the extent of liver disease, and identify vascular invasion, extrahepatic disease, and lung metastases. HB appears as a heterogeneously enhancing well-circumscribed lesion with occasional calcifications. A biopsy is usually required to confirm diagnosis before start of neoadjuvant chemotherapy and for prognostication.

Staging and Prognostication

The Children's Hepatic tumors International Collaboration (CHIC) included over 1600 children from 4 registries to investigate the predictors of poor outcomes. Age over 8 years at diagnosis, AFP less than 100 ng/ml, multifocal tumor, tumor rupture, portal and hepatic venous involvement, and metastatic disease were identified as poor prognostic markers [8].

Several staging systems have been developed to tailor treatment for HB. The preoperative extent of tumor (PRETEXT) system based on preoperative imaging, which was developed by the International Childhood Liver Tumor Strategy (SIOPEL) Group, is commonly used (Table 72.5, Fig. 72.2). This is an anatomical classification focusing on the extent of tumor and the amount of liver that can be spared during resection [9]. Modifications to the PRETEXT scoring system have included additional subclassifications to identify high-risk factors such as vascular involvement, caudate lobe, lymph node involvement, and metastatic disease (Table 72.6).

SIOPEL also classifies HB into standard-risk, high-risk, and very-high-risk groups based on the PRETEXT staging and additional factors to tailor management (Table 72.7).

Table 72.5 PRETEXT staging and frequency of various PRETEXT stages at initial presentation. Refer to Fig. 72.2

PRETEXT staging	Definition	Frequency at presentation (%)
I	One section is involved and three adjoining sections are free	4.8
II	One or two sections are involved, but two adjoining sections are free	36.6
III	Two or three sections are involved and no two adjoining sections are free	38.8
IV	All four sections are involved	19.8

Management

Early results of HB with surgical resection alone were poor due to the advanced stage at which these tumors usually present. Only 5% of all tumors at presentation can be staged as PRETEXT I, and over 50% are unresectable at initial presentation (Table 72.5). Metastatic disease in the lungs at presentation is not uncommon. However, this should not dissuade the clinician from aiming for cure. Resection of lung metastases along with treatment of primary has resulted in long-term survival [10].

HB are highly chemosensitive tumors and respond well to platinum compound-based chemotherapy. Use of chemotherapy in conjunction with surgery has radically altered the outcomes of these tumors. Today, multimodality treatment with surgery and chemotherapy is the mainstay of treatment for HB.

Ideal treatment strategy for PRETEXT I tumors is controversial. Some authors have suggested complete resection alone without chemotherapy as an option. This holds true for low-risk tumors with fetal histology where complete surgical resection with follow-up has been shown to be effective in providing long-term disease control [11]. Advocates of this approach highlight the fact that these children are spared the adverse effects of chemotherapy such as ototoxicity [12]. The SIOPEL group, however, advocates neoadjuvant chemotherapy for all HB. The purported advantages of this approach are that it shrinks the tumor and clearly demarcates the tumors making resection more straightforward. Tumors also become more fibrotic and hence intraoperative handling is easier.

The current protocol for PRETEXT II and III is to give up to four cycles of neoadjuvant chemotherapy, followed by assessment for resection (Fig. 72.3). If the tumor becomes resectable, then surgery is followed by two more cycles of chemotherapy. If the tumor remains unresectable, two further cycles of chemotherapy may be considered before attempting resection or transplantation. Additional cycle of chemotherapy has not been shown to improve outcomes but is associated with drug toxicity and drug resistance. Monitoring the fall in AFP level after beginning chemotherapy and after surgery is an excellent means of predicting tumor response. The chemotherapy regimen advised by the SIOPEL group varies for the standard-risk and high-risk HB (Table 72.7) [9].

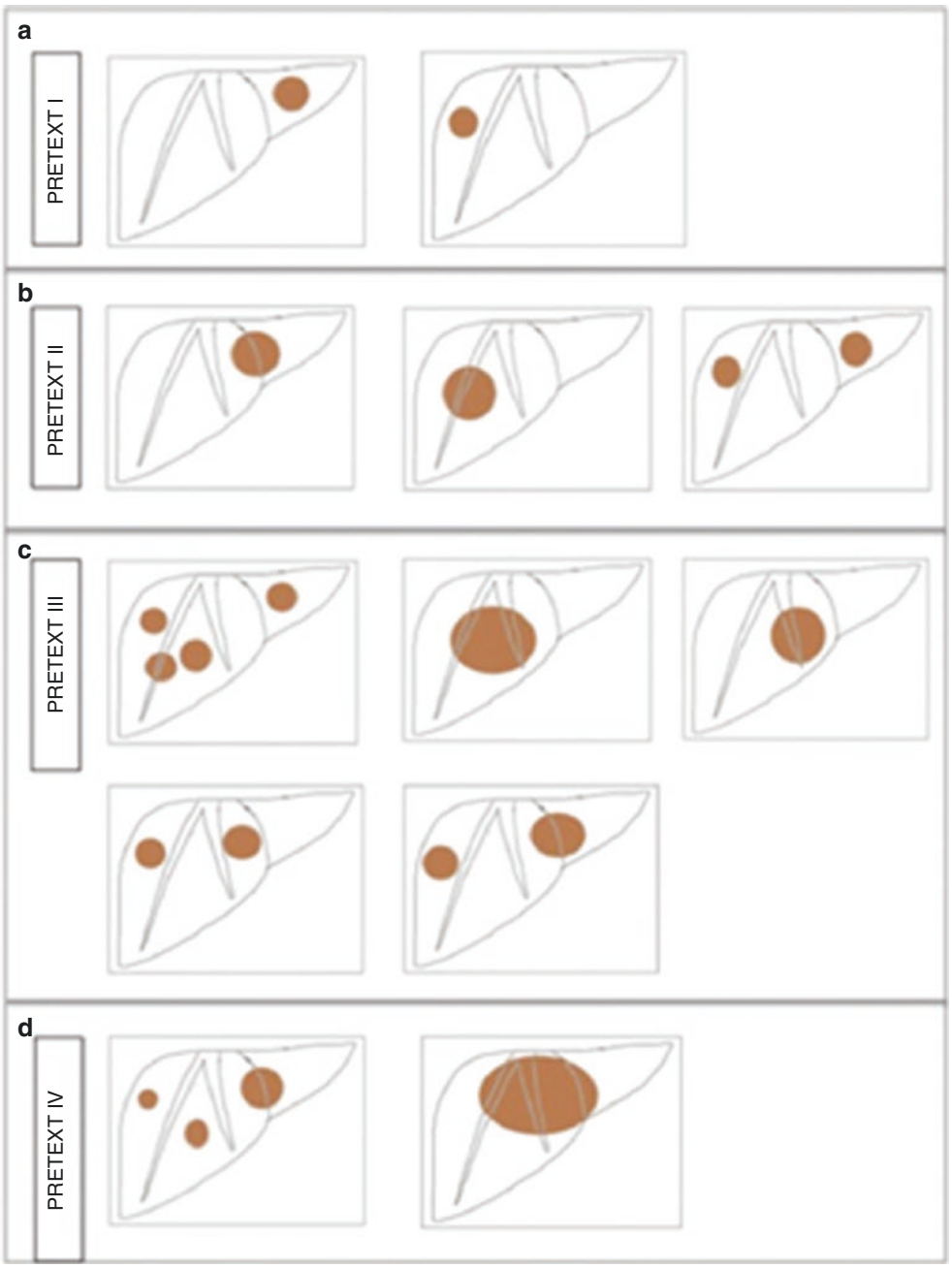

Fig. 72.2 PRETEXT staging: The PRETEXT system is based on the preoperative imaging and is an assessment of the liver that is free of tumor. The liver is divided into four sectors by the right and middle hepatic veins and the falciform ligament. The four sectors are the left lateral, left medial, right medial, and right lateral sectors. The number of contiguous sectors that are free of tumor is the key to the staging. Tumor may be single or multiple. (**a**) PRETEXT 1, three contiguous sectors are tumor-free, (**b**) PRETEXT 2, two contiguous sectors are free of disease, (**c**) PRETEXT 3, only one sector is free, (**d**) PRETEXT 4, all four sectors are involved

Surgical resection for HB should be carefully planned and is best carried out in units with expertise in pediatric hepatobiliary surgery and liver transplantation (LT). This is particularly true in children with large tumors and borderline resectability. Children can tolerate extensive liver resections better than adults, and up to 85% of liver can be resected safely. More aggressive liver resection techniques such as total vascular exclusion and caval resection may be required to achieve complete disease clearance.

LT for unresectable HB is a well-defined indication [13]. These could be PRETEXT IV tumors (solitary or multifocal), centrally located PRETEXT II or III, or tumors with portal or caval involvement (Fig. 72.4). Primary LT in high-risk cases has better outcomes than rescue transplantation after resection, and the latter should be considered a relative contraindication [14]. A recent US study reported that over the past 20 years, the percentage of HB receiving transplantation has increased from 5% to 20%, and the number of transplantations for HB has increased almost 20-fold [15]. This could be a reflection of the increasing confidence among clinicians for this radical modality of treatment.

The options are either split-liver deceased donor LT (DDLT) or living donor LT (LDLT), and both have comparable results. Children planned for DDLT usually complete

Table 72.6 Additional criteria for PRETEXT staging

Caudate lobe involvement	C1—tumor involving caudate lobe	–
	C0—all other patients	
Extrahepatic abdominal disease	E0—no evidence of tumor spread in the abdomen (except M or N)	Add suffix "a" if ascites is present
	E1—direct extension of tumor into adjacent organs or diaphragm	
	E2—peritoneal nodules	
Tumor focality	F0—solitary tumor	–
	F1—two or more discrete tumors	
Tumor rupture	H1	–
Distant metastases	M1	–
Lymph node metastasis	N0—no nodal metastases	–
	N1—abdominal lymph node metastases only	
	N2—extra-abdominal lymph node metastases	
Portal vein involvement	P1—involvement of left or right branch of portal vein	Add suffix "a" if intravascular tumor is present
	P2—involvement of the main portal vein	
IVC or hepatic vein involvement	V1—involvement of one hepatic vein, IVC free	Add suffix "a" if intravascular tumor is present
	V2—involvement of two hepatic veins, IVC free	
	V3—involvement of all three hepatic veins and/or IVC	

IVC inferior vena cava

Table 72.7 Risk stratification and treatment of hepatoblastoma (SIOPEL guidelines)

Risk status	Definition	SIOPEL guideline for treatment
Standard risk	PRETEXT I, II, III without any other risk factors as defined below[a]	*SIOPEL 3, cisplatin-alone arm*
		Cisplatin × four cycles
		Surgical resection
		Cisplatin × two cycles
High risk	PRETEXT IV or any PRETEXT stage with vascular involvement (P2 or V3), extrahepatic disease (E1, E2), tumor rupture (H1)	*SIOPEL 3, SUPERPLADO arm*
		Alternating cycles of cisplatin and carboplatin + doxorubicin × seven cycles
		Resection/transplantation
		Alternating cycles of cisplatin and carboplatin + doxorubicin × three cycles
Very high risk	Any tumor with metastases or very low AFP (<100 ng/ml)	SIOPEL 4, dose-dense cisplatin-based chemotherapy or enrolment in clinical trial

Available at www.siopel.org [9]
AFP alpha-fetoprotein, *PRETEXT* preoperative extent of tumor
[a]PRETEXT I with fetal histology may be considered for surgical resection alone and observation

neoadjuvant chemotherapy and are placed on the waiting list with some form of prioritization to enable timely transplantation. Where LDLT is an option, transplantation can be planned within 4–6 weeks of completing neoadjuvant chemotherapy. Transplantation should be followed by adjuvant chemotherapy. Presence of lung metastases increases the risk of posttransplant recurrence but is not a contraindication for LT as long as they can be completely resected [15]. Close follow-up in the posttransplantation period is essential and usually involves periodic AFP estimation and imaging studies.

Overall disease-free survival has consistently improved for HB with multidisciplinary management using chemotherapy, LT, and radical resection [16]. Low-risk tumors have a 90% 5-year survival, while high-risk tumors have a survival of around 50%. Histological subtype has an impact on survival. Fetal types of HB have the best outcomes, while the small-cell undifferentiated type of tumors have worse prognosis. Relapse after initial complete response occurs in 11% of children. The liver and lungs are the commonest sites of relapse. Positron emission tomography/computed tomography (PET/CT) has been reported to be better at identifying relapse when compared to CT or MRI [17]. A combination of surgery and chemotherapy provides the best chance of achieving a second complete response in these children with reported 3-year overall survival of over 40% [18].

Hepatocellular Carcinoma (HCC)

HCC is the second most common liver tumor in children. The incidence is bimodal, with an early peak that is lower than that of hepatoblastoma. Most of these early cases occur before 5 years of age. A second peak occurs between 13 and 15 years of age. These form 15% of all malignant liver tumors in children and 75% of all liver tumors in adolescents. HCC in children can occur in a background of chronic liver disease secondary to hepatitis B or metabolic disorders, though non-cirrhotic HCC is more common than in adults. Other conditions associated with the development of HCC include cirrhosis, α1-antitrypsin deficiency, tyrosinemia, aflatoxin ingestion, hemochromatosis, hepatic venous obstruction, androgen and estrogen exposure, Alagille syndrome (arteriohepatic dysplasia), and Thorotrast administration. With increasing use of universal hepatitis B immunization, the incidence of HCC in children has decreased [19].

HCC usually presents as an abdominal lump (40%). Occasional presentation may be with abdominal pain or jaundice due to biliary compression or tumor ingrowth into the biliary tree. Rarely, these tumors may present as an emergency due to bleeding or rupture. HCCs are usually associated with an elevated AFP level though the elevation is not as

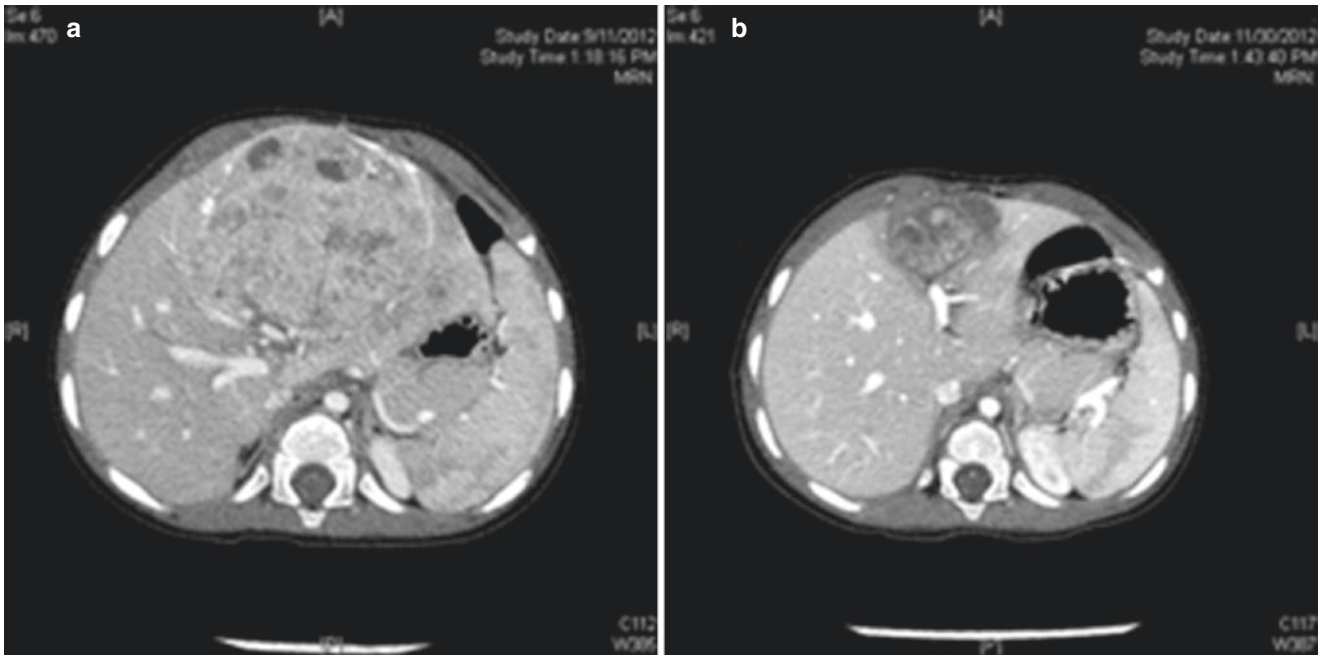

Fig. 72.3 CT images of hepatoblastoma in a 1-year-old child. (**a**) CT at presentation showed a PRETEXT 2 disease with tumor involving the two left sectors. (**b**) CT following four cycles of chemotherapy prior to resection. Note the significant shrinkage in tumor size. The CT appearance of the tumor has also changed, and the demarcation between the tumor and healthy liver tissue is much more clear

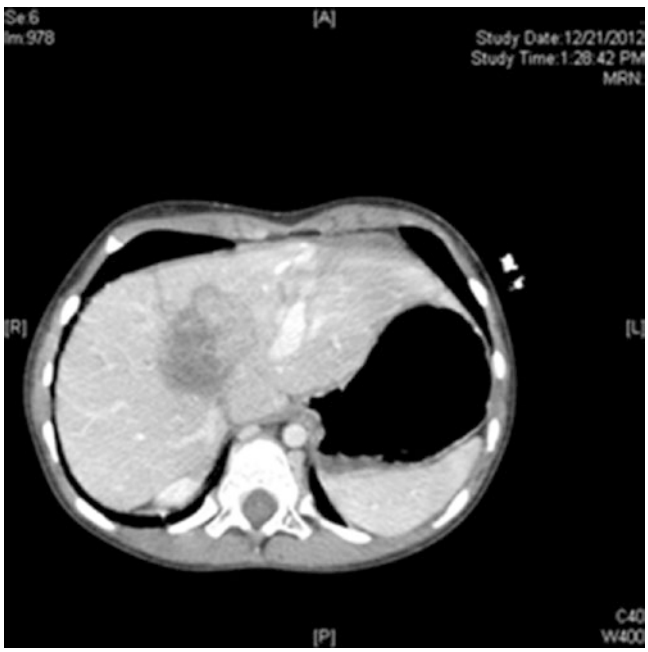

Fig. 72.4 CT image of a hepatoblastoma in a 6-year-old child. This child had persistent PRETEXT III tumor after six cycles of chemotherapy. The tumor was closely approximated to the cava. She underwent primary living donor liver transplantation

high as that seen in HB. Liver function tests should be evaluated to assess the severity of any underlying liver disease. Metabolic screen is important to identify underlying liver disease which may impact management. Triphasic CT or MRI with contrast can help in characterizing the lesion, presence of chronic liver disease, any vascular involvement, and the presence of metastases.

HCCs are chemoresistant tumors, and complete resection provides these children with the best chance of long-term cure. However, the feasibility of resection depends on the size and extent of the tumor and the presence of underlying liver disease. In patients with underlying chronic liver disease, LT has the benefit of treating the underlying liver disease and providing adequate tumor clearance. Trans-arterial chemoembolization has been used as a bridge or downstaging therapy in children with HCC awaiting LT [20].

In the adult setting, the option of LT is available only for HCC within strict size and number criteria [21]. It is unclear if these criteria are appropriate in the pediatric setting, as most children will present with large tumors. Studies have shown good results in children transplanted for HCC beyond Milan criteria [22]. Hence, unless there is major vascular invasion or extrahepatic disease, unresectable HCC in children should be considered for LT. Presence of lung metastases is a contraindication for LT in pediatric HCC unlike HB due to the high risk of posttransplant progression of metastatic disease. Use of sorafenib in combination with cisplatin-based chemotherapy has also been reported in the management of pediatric HCC [23]. Outcomes of pediatric HCC are inferior to HB. Recurrent disease is the most common cause of death in these children and usually occurs in the lungs or bones.

Fibrolamellar Hepatocellular Carcinoma

This is an uncommon primary liver tumor seen in older children and young adults. The median age at presentation is 21 years. It is less common than standard HCC and occurs on a background of a non-cirrhotic liver. An elevated AFP is only seen in around 10% of these tumors. They are detected incidentally when they become symptomatic and are hence large at presentation. In a systematic review of available literature of fibrolamellar HCC (FL-HCC), the mean size of tumor at surgery was 12 cm [24]. On CT imaging, they appear as large well-circumscribed lesions which enhance strongly in arterial and portal venous phases becoming isodense in delayed scans. A poorly enhancing central scar may be seen. These tumors spread by both lymph node and blood-borne systemic metastases.

Surgical resection offers the best chance of cure for these tumors with a 5-year survival of 70% in adults [25]. However, within the pediatric age group, results of FL-HCC do not appear to be superior to standard HCC because of late presentation and local recurrence after resection [26]. LT for FL-HCC has been reported though the long-term results are not encouraging possibly because of large tumor size at presentation and presence of lymph nodal metastases.

Transitional Liver Cell Tumors

These are a special group of tumors initially described by Prokurat et al. that share characteristics of both HB and HCC [27]. They are purported to be a subset of liver cell tumors in older children and adolescents that evolve along a transition pathway between blastomatous tumors and adult-type tumors. AFP levels are elevated only in 40–60% of patients, and this elevation is at least one log less than what is observed in HB. Histologically, they have features intermediate between the macrotrabecular variant of HB and trabecular HCC. Most of these tumours are initially diagnosed as HB and treated as such. Liver biopsy may fallaciously allow for a diagnosis of pure HB due to sampling errors which may miss islands of HCC. They have a much poorer outcome which is more in keeping with HCC than HB.

Embryonal Sarcoma (Undifferentiated Sarcoma of the Liver)

This is a rare mesenchymal tumor constituting 5% of all liver tumors in children. The median age of presentation is between 6 and 10 years and is more common in males. There have been several reports of development of embryonal sarcoma from mesenchymal hamartomas [28]. Studies have also shown similar karyotypic abnormalities in both tumors. Presentation is with an abdominal lump or pain. This tumor has characteristic radiological appearance. Ultrasonography (USG) shows a solid isoechoic clearly demarcated tumor. CT shows a well-circumscribed hypoattenuating lesion with multiple enhancing septations. An enhancing pseudocapsule may be present. The tumors are usually large at presentation and may be associated with lung metastases. Histologically, these tumors demonstrate large areas of necrosis with patches of viable tumor. Stellate or spindle-shaped tumor cells are loosely arranged in a myxoid matrix (Fig. 72.5).

Initial reports of this tumor described poor prognosis [29]. However, recent reports have shown that multimodality approach combining resectional surgery, chemotherapy, and transplantation can provide survival rate of up to 90% [30, 31].

Biliary Rhabdomyosarcoma

This is the most common cause of malignant biliary obstruction in the pediatric age group. These tumors arise from any part of the intrahepatic and extrahepatic biliary tree including the gallbladder and ampulla of Vater. The median age at presentation is 3 years, and children commonly present with jaundice and abdominal pain. Preoperative diagnosis is usually a choledochal cyst [32]. CT scan shows a dilated biliary tract with a hypoattenuating tumor in the bile duct. Surgery helps in confirming diagnosis and enables resection of the tumor and biliary drainage in the form of a hepaticojejunostomy. Surgical excision with negative microscopic margins is rarely possible. These tumors have been found to respond

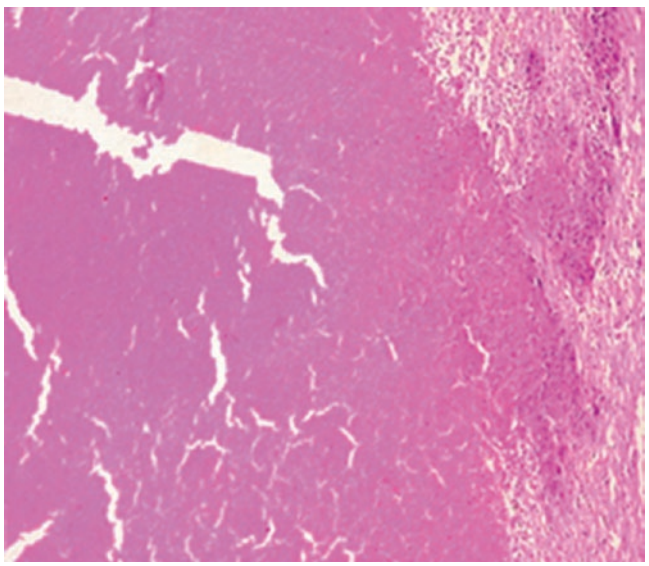

Fig. 72.5 Histology of embryonal sarcoma. Tumor shows large areas of necrosis with islands of spindle-shaped tumor cells in a myxoid matrix

well to adjuvant multi-agent chemotherapy [33]. Spunt et al. reported 25 cases of biliary rhabdomyosarcoma with a 5-year survival of 66% for all cases and 78% for children without systemic metastases. Death was primarily due to recurrent disease or complications of aggressive resection and/or chemotherapy [34].

Calcifying Nested Stromal–Epithelial Tumor

These are rare primary liver tumors of uncertain histogenesis that typically occur in children and young adults. Histologically, they have a characteristic appearance with circumscribed nests and islands of bland-appearing epithelioid cells. Focal psammoma-like calcifications with or without ossification may be present. The tumors typically have an indolent course and are considered as low-grade malignancies. Surgical resection is the treatment of choice. Some of these tumors have been reported to be associated with Cushing's syndrome which improved on resection [35, 36].

Hepatic Angiosarcoma

These are highly malignant vascular tumors with a uniformly poor prognosis. There are reports of malignant transformation of multifocal or diffuse hemangioma into hepatic angiosarcoma [37]. Differentiation from diffuse hepatic hemangioma or hemangioendothelioma may be difficult preoperatively on imaging alone, and clinical presentation can provide clues toward the correct diagnosis. Complete resection is required for cure of angiosarcoma, but recurrence rates are high, and the tumor is often not resectable; it is typically present in both lobes of the liver, and consideration should be given to adjuvant chemotherapy. LT should be avoided due to early development of systemic metastases [38]. Some authors have suggested a waiting time of 6 months in patients with inconclusive preoperative imaging to assess the natural history of these tumors to avoid performing a futile LT [39].

Benign Liver Tumors

Hemangioma

HMGs are the most common benign tumors in the pediatric setting. They constitute over half of all liver tumors in the neonatal period. The Liver Hemangioma Registry of the Vascular Anomalies Center at the Children's Hospital of Boston recognizes three clinical subgroups of infantile hepatic HMG—focal, multifocal, and diffuse [40]. Focal HMGs are usually single and indolent. Their clinical presentation is based entirely on their size and usually present as a large abdominal mass. Smaller focal lesions are diagnosed only on routine imaging studies in the antenatal period or after birth and usually have no clinical relevance. Associated extrahepatic lesions are uncommon with focal HMGs. Multifocal HMGs present as multiple lesions in one or both lobes of the liver or may involve the liver diffusely. Histologically, they may be classified as capillary or cavernous HMG and may rarely show pleomorphism, intravascular spread, necrosis, and hemorrhage. Over half of these children have extrahepatic vascular lesions, most commonly in the skin but also in the brain, eye, etc. Status of the cutaneous lesions has been used as a marker for monitoring the involution of the liver lesions [41].

Abdominal lump is the usual presentation. High-output cardiac failure and consumptive coagulopathy due to intratumoral trapping and destruction of platelets causing a disseminated intravascular coagulopathy like picture are rare but serious complications [42, 43]. Abnormalities in thyroid function have been reported in these children [44]. There appears to be an overlap in clinical behaviors of diffuse hepatic hemangiomas and hepatic angiosarcoma [45]. Multifocal or diffuse hemangiomas may be difficult to differentiate from malignant vascular tumors.

Treatment of these lesions should be tailored to individual presentation [41]. Small, asymptomatic lesions are best left alone. Symptomatic lesions have been treated with a variety of pharmacological and interventional approaches. Steroids, chemotherapeutic agents, β-blockers, hepatic artery ligation, and hepatic artery embolization have all been reported by various authors to be useful. Large symptomatic lesions need liver resection and are best managed in centers with advanced hepatobiliary and LT expertise. LT has been reported in large symptomatic lesions not amenable to resection [41].

Hemangioendothelioma

Hemangioendotheliomas are highly proliferative cellular lesions of variable malignant potential. The incidence is 1%, and it is the most prevalent hepatic vascular tumor in pediatric patients younger than 6 months [44]. Infants with large, actively perfused vascular lesions may come to medical attention with congestive heart failure [42, 43]. Angiosarcomatous transformation in a specific type of these tumors (type 2 hemangioendothelioma) or a coexisting malignant component within a hemangioma-like tumor has also been reported [37]. These lesions may appear very cellular, but they do not metastasize. If a primary lesion produces symptoms, resection is indicated for relief. For lesions which are unresectable, LT may be offered as the treatment of choice [44].

Mesenchymal Hamartomas

These rank second in frequency among benign liver neoplasms in children. They usually present as a large abdominal mass in the first 2 years of life. Some of these tumors may be detected incidentally or because of symptoms of abdominal pain, vomiting, or failure to thrive. Rare presentations are with respiratory distress, high-output cardiac failure in the newborn, and jaundice, hemorrhage, or rupture [46].

Imaging usually shows a single tumor in the right lobe with solid and cystic components. Occasionally, one cyst may be large giving it the appearance of a single cystic lesion. Microscopic examination shows varying proportions of epithelial and loose connective tissue components arranged in a disorganized pattern. The epithelial element consists of bile ducts and hepatic parenchyma without acinar pattern. Numerous arteries and veins are scattered in the loose myxoid stroma. Cystic degeneration and foci of extramedullary hematopoiesis are also identified. Some authors have classified it as a neoplasm rather than a hamartoma. Reports that these tumors are associated with karyotype abnormalities such as aneuploidy and balanced translocations suggest a malignant potential [28].

Treatment is primarily surgical. Complete excision is recommended, and, where not possible, LT has been reported [46].

Hepatic Adenoma

Hepatic adenomas are observed in children with type I glycogen storage disease after the first decade of life and may be multiple. Hepatic adenomas are known to occur in patients undergoing androgen therapy for hematologic disorders [47, 48]. These are diagnosed in older children and adolescents and usually identified on routine screening, though bleeding and rupture of superficial adenomas may lead to a more acute presentation [47, 48]. Hepatic adenomas may show encapsulation on imaging studies, but histologic sections may be difficult to distinguish from HCC. It is best to remove adenomas because of the difficulty of differentiating them from low-grade HCCs. There also remains the uncertainty about future malignant degeneration, and the possibility of rupture and hemorrhage. Small adenomas may however be followed up by serial imaging, but larger adenomas or superficial adenomas presenting with pain should be resected to avoid complications [47, 48].

Focal Nodular Hyperplasia

FNH is uncommon in children and constitutes about 5% of all pediatric liver tumors. An association with previous bone marrow cancers, bone marrow transplantation, or congenital or surgical portosystemic shunts has been reported [48, 49]. A possible response of the liver tissue to the cytotoxic chemotherapy has been postulated. FNH has been reported in an infant antenatally exposed to corticosteroids. Similarly, borderline ischemia of the liver due to the shunting has also been postulated as a possible explanation of their coexistence with shunts. The most common presenting symptom is an incidentally detected mass in the abdomen. FNH is usually diagnosed on routine imaging and is identified as a slightly hypodense, discrete lesion on plain CT. On contrast images, it enhances homogeneously in the arterial phase with the lesion becoming isodense in delayed scans. A central scar is noted in 50% of cases with delayed enhancement of the central scar being a characteristic finding.

Macroscopically, it appears as a well-circumscribed mass with a central depression (Fig. 72.6). Cut surface shows small nodules divided by fibrous septa leading to a central scar. Microscopically, the nodules contain hyperplastic hepatocytes supported by a well-developed reticulin framework. The septa contain abundant vessels, consisting of both arteries and veins [50, 51]. Variable eccentric intimal fibroplasia and disruption of the elastic lamina are noted in the arteries. Inflammatory cells and numerous proliferating ductules are also identified in the septa. Chronic cholestatic features are also identified in the cells adjacent to septa.

These lesions may be small and multiple or may be single and large. If imaging is suggestive of FNH, then close follow-up with serial imaging to detect any increase in size is recommended. Superparamagnetic oxide-enhanced MRI may discriminate between hepatic adenoma and FNH. Biopsy may also be necessary in doubtful cases, but might be prone to sampling errors. Asymptomatic cases of FNH are observed using serial abdominal US, MRI, and clinical examination. Spontaneous regression has been documented. Apart from large, symptomatic lesions, surgery is indicated where there exists a diagnostic dilemma of differentiating the lesion from an adenoma [51, 52].

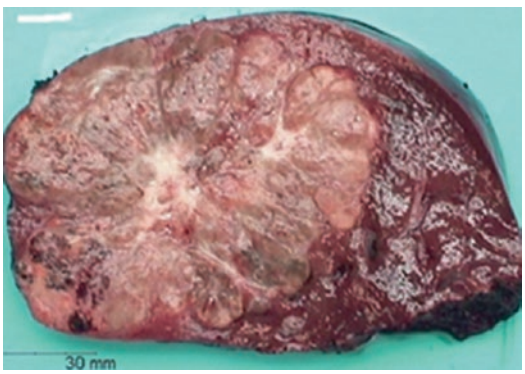

Fig. 72.6 Cut section of focal nodular hyperplasia. Note the well-circumscribed lesion with multiple nodules separated by fibrous septa joining at a central scar

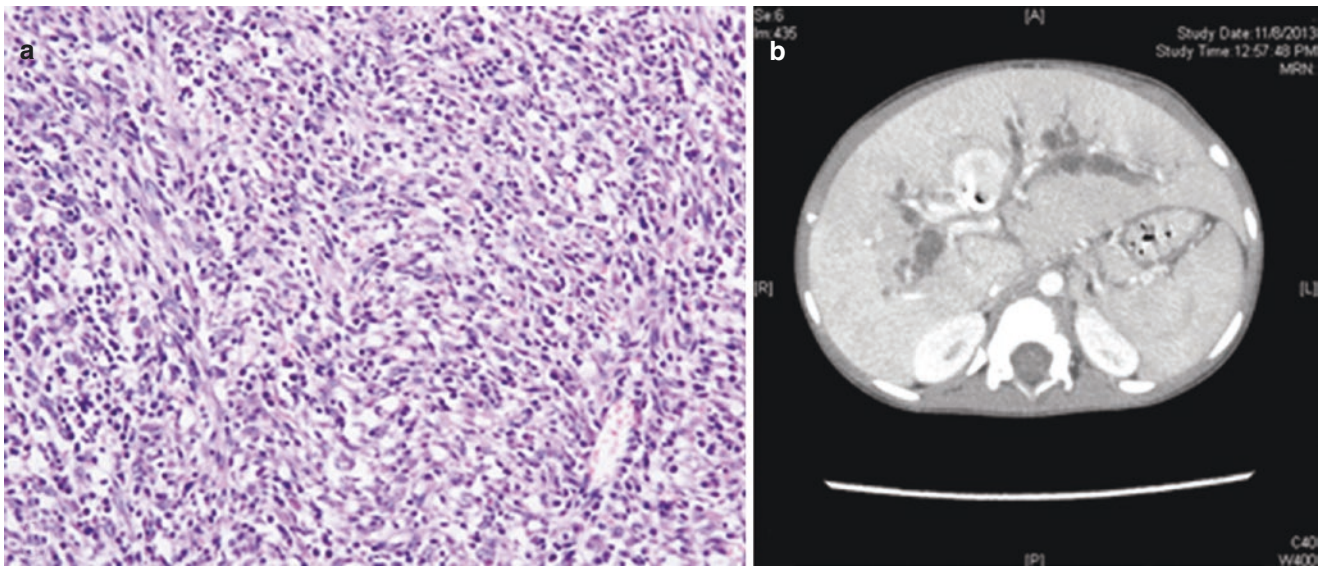

Fig. 72.7 (**a**) Section of an inflammatory myofibroblastic tumor displaying interlacing bundles of fibroblasts admixed with inflammatory cells. (**b**) CT showing an inflammatory myofibroblastic tumor involving the hepatic hilum in a 4-year-old child. The child presented with constitutional symptoms, obstructive jaundice, and recurrent episodes of cholangitis. He underwent endoscopic stenting with no relief of symptoms. Surgical resection (extended right hepatectomy with excision of extrahepatic biliary tract) was carried out

Inflammatory Myofibroblastic Tumor

Inflammatory myofibroblastic tumors (IMFT) are predominantly benign lesions of unknown origin. These tumors can occur anywhere in the body and have occasionally been reported in the liver where they may mimic liver tumors.

These lesions have a characteristic histological picture comprising spindle-cell proliferation admixed with chronic inflammatory cell infiltrate of plasma cells, lymphocytes, and histiocytes (Fig 72.7a). Anaplastic lymphoma kinase gene rearrangements have been noticed in about half of IMFT, further supporting its neoplastic nature. Expression of anaplastic lymphoma kinase is associated with localized disease at presentation and an improved prognosis [53].

Presentation may be with constitutional symptoms such as fever, jaundice, and weight loss. Their natural history is variable with some reports of spontaneous regression, while some children may develop recurrent episodes. Local invasion has been reported in some cases (Fig. 72.7b). Surgical resection is indicated if the lesion persists or progresses after a trial of conservative therapy, or manifests evidence of local infiltration into vital structures, or of malignant transformation. Complete resection is curative in most patients [54].

Secondary Liver Lesions

The liver is a relatively frequent site of metastatic disease in childhood. Non-Hodgkin lymphoma, neuroblastoma, rhabdomyosarcoma, Wilms' tumor, adrenal cortical carcinoma, osteogenic sarcomas, and a host of other malignancies may metastasize to the liver [55–58]. These are usually multiple and are best managed with multimodality therapy. Criteria for surgical removal of hepatic metastases include control of the primary site, a solitary or limited number of metastases, adequate hepatic reserve, good performance status, and a reasonable expectation of prolonged survival or cure [55–58]. Resecting liver metastases from neuroblastoma or Wilms' tumor have the advantage of diminution of treatment and a prolonged survival [57, 58].

Conclusion

Liver tumors in children are uncommon. Most present as an abdominal lump and are usually advanced at initial presentation. HB is the most common childhood liver tumor. Evidence-based multimodality therapy using improved liver resection techniques, LT, and use of effective chemotherapeutic agents has improved outcomes in these tumors.

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Acute Liver Failure in Children

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Naresh P. Shanmugam and Anil Dhawan

Introduction

Acute liver failure (ALF) used synonymously with fulminant hepatic failure is a blanket term used to describe severe and sudden onset of liver cell dysfunction leading to synthetic and detoxification failure across all age groups. Pathogenesis of hepatocyte cell injury is multifactorial (Fig. 73.1) and liver failure is the outcome of a complex equation between hepatocyte death and regeneration, as suggested by the US Acute Liver Failure Study Group [1]. Injured liver cells secrete several bioactive substances and toxins that initiate cascade of events leading to multiorgan failure (Fig. 73.2).

Definition

"Fulminant liver failure" was coined by Trey and Davidson 40 years ago to define onset of hepatic encephalopathy (HE) within 8 weeks of appearance of symptoms of liver dysfunction in an otherwise healthy individual with no prior history of liver disease [2]. O'Grady and colleagues categorized ALF as "hyperacute (1 week), acute (1–4 weeks), and subacute failure (5–12 weeks)" depending on the interval between jaundice and onset of encephalopathy, for prognostication purpose [3]. It is difficult to use adult-based definitions in children, as encephalopathy is a late sign and sometimes ALF has in utero onset and so time quantification would be difficult. Acknowledging the fact that diagnosing encephalopathy in children is difficult and often a late and terminal event, Bhaduri and Vergani proposed the definition of ALF as "A rare multisystem disorder in which severe impairment of liver function with or without encephalopathy, occurs in association with hepatocellular necrosis in a patient with no recognized underlying chronic liver disease" [4]. Even this definition does not seem to be clear as the term "severe impairment of liver function" in the definition did not have any objective quantification values of liver function and so there was a lot of subjective variation in diagnosing ALF.

The Pediatric Acute Liver Failure Study Group (PALFSG) developed a working definition to identify ALF in children without interobserver variation. The PALFSG used international normalized ratio (INR) in the background of acute liver disease as an objective measurement to demarcate acute hepatitis and ALF. As per PALFSG, ALF is defined as (i) hepatic-based coagulopathy defined as a prothrombin time (PT) 15 s or INR 1.5 not corrected by vitamin K in the presence of clinical HE or a PT 20 s or INR 2.0 regardless of the presence or absence of clinical HE, (ii) biochemical evidence of acute liver injury, and (iii) no known evidence of chronic liver disease [5]. Asymptomatic preexisting liver disease, which manifests acutely, should be considered as ALF, if they fulfill the diagnostic criteria, as in acute fulminant Wilson's disease.

Coagulopathy is not only a key criterion in diagnosing pediatric ALF but also acts as a prognostic marker. Due to short half-life of several liver-based clotting factors, PT/INR functions as a dynamic marker of synthetic inadequacy due to loss of liver cells in ALF. Factors II, VII, IX, and X depend on vitamin K to convert them into active form. Correction of coagulopathy by intravenous vitamin K differentiates between vitamin K deficiency due to decreased absorption from liver synthetic failure. Isolated prolonged APTR is not due to liver disease, as factor VII in extrinsic pathway (Fig. 73.3) has the shortest half-life (4–6 h) of the vitamin K-dependent factors, which therefore is the first factor depleted in ALF and invariably affects INR. Coagulopathy secondary to disseminated intravascular coagulation (DIC) precipitated by infection is common in acute liver injury, and so it is essential to rule out DIC before making a diagnosis of ALF based on INR.

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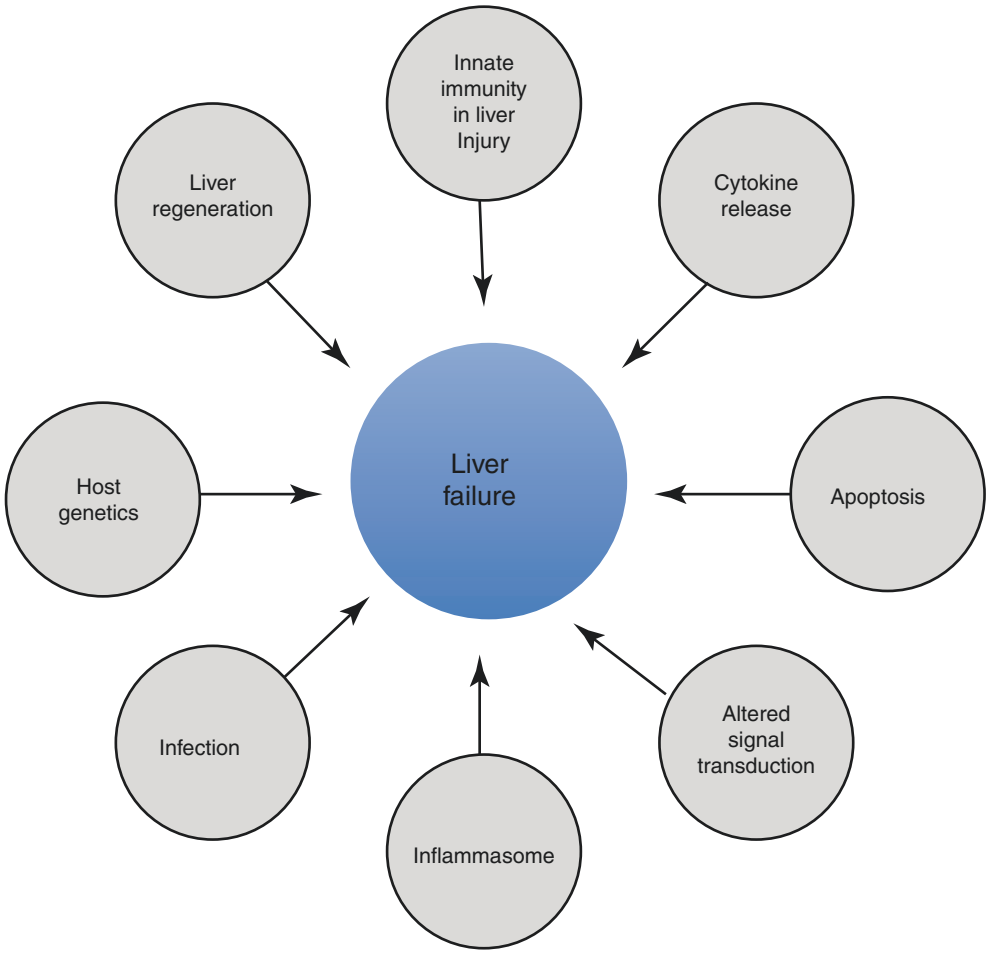

Fig. 73.1 Pathogenesis of liver cell injury is multifactorial with several factors influencing liver cell death and regeneration. When the net result is liver cell loss beyond a critical mass required maintain normal body function, liver failure sets in

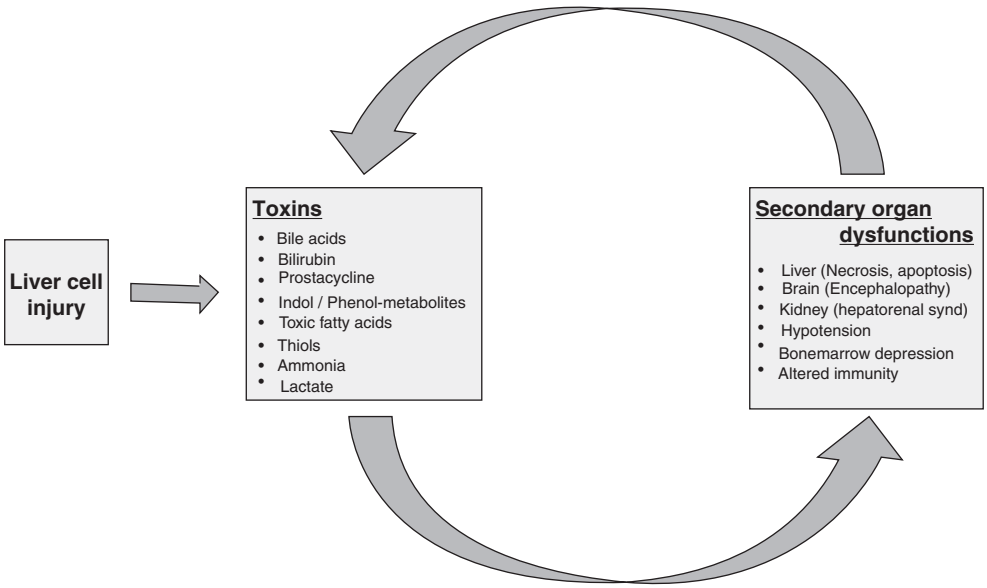

Fig. 73.2 Except for Von Willebrand factor and tissue plasminogen activator, the liver is the major site for synthesis of clotting factors. Factors V synthesis is first to be affected, and factor VII has the shortest half-life and is theoretically more sensitive marker than INR of hepatic synthetic function. *INR* international normalized ratio

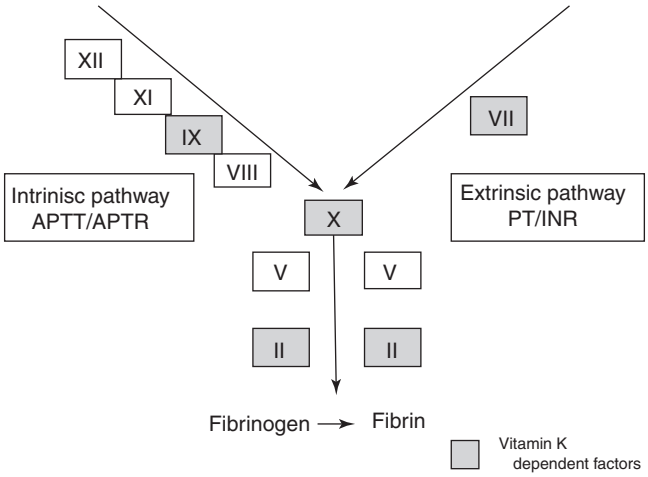

Fig. 73.3 Liver cell injury leads to release several toxic substances that cause secondary organ dysfunction; this leads on to a vicious cycle of autointoxication. APTT, activated partial thromboplastin time, APTR, activated partial thromboplastin ratio, *PT*, prothrombin time, *INR*, international normalized ratio

Etiology

The etiologies of ALF differ within age groups and geographic location. In Southeast Asia and Latin America, viral hepatitis A and E are the most common cause of ALF in children, while in Northern America and Europe, etiology remains elusive (indeterminate) in majority of children [6]. Certain disorders, such as neonatal hemochromatosis, are very unique to pediatric population. The exact incidence of ALF in pediatric age group is not known, but probably the overall annual incidence of ALF in USA is around 5.5/million population among all ages [7].

Infection

Hepatitis A and E viral (HAV and HEV) infections are the common cause of ALF in developing countries with poor sanitation and overcrowding, as these viruses are spread by contaminated water and food. The risk of liver failure is 0.1–0.4% following symptomatic HAV infection, and it increases with a preexisting liver disease. Specific diagnosis is established by detecting HAV immunoglobulin (Ig) M antibodies in the blood at presentation. The infection is most often self-limiting with subsequent recovery and only in few it might be severe enough requiring liver transplantation [8]. The risk of developing ALF in adults following HEV infection is 0.6–2.8%, with higher risk if contracted during pregnancy [9]. Bhatia et al. showed that the case-fatality associated with HEV-induced ALF in pregnancy is similar to that of age-matched general population [10].

The ALF due to hepatitis B virus (HBV) can occur at the time of acute infection, reactivation of chronic HBV infection, or seroconversion from a hepatitis Be antigen-positive to a hepatitis Be antibody (HBeAb)-positive state. Infants born to HBeAb-positive mothers are at special risk and could present with ALF around 6 weeks to 9 months [11]. Super infection or coinfection of hepatitis delta virus (HDV) in HBV-infected patients can cause liver failure. Hepatitis C viral (HCV) infection has not been reported as a cause of ALF.

Herpes simplex viruses 1 and 2 (HSV) are the predominant cause of viral-induced ALF during the first month of life. Neonatal ALF due to HSV carries a high mortality of about 85% and should be suspected in any neonate with or without vesicular rash who is unwell with dramatically high transaminases and coagulopathy. Treatment with high doses of acyclovir should be initiated in all infants with ALF, while awaiting serology results as the associated liver failure is rapidly fatal. Liver transplantation has a good outcome when considered in a hemodynamically stable neonate with ALF due to HSV [12]. Other members of herpes virus family such as cytomegalovirus, Epstein–Barr virus, and varicella-zoster virus can cause ALF. Rarely, viruses such as Dengue, Lassa, Ebola, Marburg, and Toga and bacteria such as *Leptospira* and *Salmonella* are implicated as a cause for ALF.

Drugs and Toxins

In developed countries, drug-induced liver failure is the most common identifiable cause of ALF in adults and children. Drug hepatotoxicity could be an idiosyncratic reaction, a dose-dependent response, or a synergistic reaction due of several drugs. It is essential to include details of complementary therapies and herbal medications as some are potential hepatotoxics [13]. Acetaminophen is the most common drug associated with ALF, probably due to the easy availability without the need for a prescription. It is safe when used in recommended doses and toxicity is usually dose dependent. Acetaminophen is detoxified mainly by glucuronidation (40%), sulfation (20–40%), and *N*-hydroxylation (15%). A small fraction is metabolized via cytochrome P450 to yield *N*-acetyl-para-benzoquinone-imide (NAPQI), a toxic intermediate compound which irreversibly conjugates with the sulfhydryl group of glutathione and causes hepatocyte necrosis [14]. Genetic polymorphism of cytochrome P450 isoenzymes predisposes affected people to acetaminophen toxicity due to increased NAPQI production. Bound NAPQI forms acetaminophen–protein adducts, which act as a specific biomarker for chronic acetaminophen toxicity. Hepatotoxicity of acetaminophen is dose dependent, and liver injury results in either because of acute higher doses of ingestion or cumulative overdoses taken over a few days. In acute acetaminophen

overdose, plotting serum levels after 4 h against time on the Rumack nomogram will guide toward potential toxicity. However, it may not be useful in situations of cumulative over doses. In such cases, bound NAPQI forms of acetaminophen–protein adducts in blood would aid the diagnosis. The mechanism of toxicity of anti-tuberculosis drugs, particularly isoniazid, is similar to acetaminophen; oxidation via cytochrome P450 pathway results in toxic metabolites.

Most common cause of idiosyncratic drug-induced liver injury (DILI) are due to antibiotics and NSAIDs [14]. The reported incidence of idiosyncratic DILI was around 14 new cases/100,000/year, of which 8% would progress to ALF [15, 16]. Genetic susceptibility of an individual and unmasking of underlying mitochondrial cytopathies by certain drugs are some of the proposed causes of DILI [17]. Drugs such as diclofenac, mefenamic acid, valproic acid, amiodarone, isoniazid, etc. can unmask mitochondrial cytopathy and cause ALF. Chemotherapy drugs are known to produce veno-occlusive disease leading to ALF due to endothelial damage.

Council for International Organizations of Medical Sciences/Roussel Uclaf Causality Assessment Method (CIOMS/RUCAM) scale is helpful in establishing causal relationship between offending drug and liver damage. Using the scoring system, suspected drug could be categorized into "definite or highly probable" (score > 8), "probable" (score 6–8), "possible" (score 3–5), "unlikely" (score 1–2), and "excluded" (score 0) [18]. This scale is helpful in identifying drug-induced hepatotoxicity even in newly marketed drugs and for a previously unreported older drug.

Metabolic Disorders

Metabolic disorders presenting as ALF is common in young children. Galactosemia and tyrosinemia 1 are the most common cause of neonatal liver failure, presenting with hepatomegaly, jaundice, and coagulopathy. Classical galactosemia is an autosomal recessive condition resulting in the mutation in galactose-1-phosphate uridyl transferase gene located on chromosome 9p13. Liver failure in this condition is thought to be due to accumulation on galactitol. Neonates presenting with hepatitis or ALF should be started on a galactose-free formula until galactosemia. Galactose-free diet coupled with supportive management helps the liver to recover, but some may still progress and need a liver transplant. Despite strict galactose-free diet, patients could develop complications like developmental delay, motor disorders, and hypergonadotropic hypogonadism due to endogenous galactose production on a long-term follow-up [19]. Tyrosinemia 1 is an autosomal recessive condition due to defect in the enzyme fumarylacetoacetate hydroxylase. This causes accumulation of intermediate compounds, maleylacetoacetic acid, and fumarylacetoacetic acid, which are then converted to succinylacetone, a toxin that damages the liver and kidneys. Tyrosinemia should be suspected when a coagulopathy and modest rise of transaminases is associated with elevated alpha-fetoprotein levels. Fructosemia and inborn errors of bile acid synthesis are a few other rare cause of ALF during infantile period.

Medium-chain acyl-coenzyme A dehydrogenases (MCAD) are group of enzymes involved in β-oxidation of 6–12 carbon chain fatty acids in mitochondria. They help in ketone production from fatty acids when hepatic glycogen stores become depleted during prolonged fasting and periods of higher energy demands. Affected children could present with hypoketotic hypoglycemia, recurrent liver failure, and precipitated by otherwise minor illness. Unless treated with dextrose supplementation, these episodes may quickly progress to coma and death.

Wilson's disease, an autosomal recessive disorder, could present as ALF. The acute hepatic presentation is usually characterized by the presence of liver failure, Coombs-negative hemolytic anemia, and low serum alkaline phosphatase. Diagnosis might be difficult as blood test might show weakly positive autoantibodies, and tissue copper estimation might not be possible due to coagulopathy. Demonstration of Kayser–Fleischer rings is diagnostic of Wilson's disease in a patient who presents with ALF. Wilson's disease presenting with ALF has high mortality without transplantation.

Mitochondrial disorders are group of spontaneous or inherited disorders of mitochondrial proteins resulting in defective oxidative phosphorylation, fatty acid oxidation, urea cycle, and other mitochondrial pathways [20]. This can affect the function of various cell types, such as neurons, myocytes, etc., where the need for energy requirement is high. Deficiencies of complex I, III, and IV, multiple complex deficiencies, and mitochondrial DNA (mtDNA) depletion syndrome are associated with liver failure. Diagnosis might be difficult due to particularly mtDNA depletion syndrome where there is tissue-specific mitochondrial enzyme deficiency. The infants usually presents with hypotonia, hypoglycemia, feeding difficulties, seizures, and deranged liver function. Liver transplantation could be offered in isolated liver-based mitochondrial disorders, while in multisystemic involvement it should be deferred; hence it is of utmost important to perform thorough investigation to rule out neuromuscular involvement [21].

Gestational Alloimmune Liver Disease

Neonatal hemochromatosis (NH) is the single most common cause of ALF during the first month of life, where there is massive iron deposition in the liver and extrahepatic tissues, but with sparing of the reticuloendothelial system. The pattern of iron overloading is similar to hereditary hemochro-

matosis, but NH affects only newborn and so far no specific genetic mutation has been identified. NH was considered to be a primary disease of iron overload of liver leading to liver failure. Under current concept, iron accumulation in NH is considered to be a secondary phenomenon due to immune-related severe fetal liver injury resulting in impaired regulation of maternofetal iron flux [22]. NH is now referred to as gestational alloimmune liver disease (GALD), as maternal antibody is directed toward fetal liver antigen resulting in activation of fetal complement leading to the formation of membrane attack complex (MAC) resulting in hepatocyte loss [23, 24]. This hypothesis is supported by successful prevention of severe disease by antenatal and postnatal treatment with intravenous Ig. GALD presents with jaundice, coagulopathy, moderately elevated alanine aminotransferase, high ferritin, and raised iron saturation levels. High ferritin is seen in other cause of neonatal liver failure, and no single biochemical test is diagnostic of NH. The diagnosis can only be confirmed by demonstration of extrahepatic iron deposits sparing the reticuloendothelial system. Labial salivary gland biopsy is a safe and effective way of demonstrating this [25]. The disease varies in severity; at one end of the spectrum, it is associated with fetal death, while at the other end, spontaneous recovery is reported.

Malignancies

Hemophagocytic lymphohistiocytosis (HLH) is a malignant disorder of the hematopoietic system where there is uncontrolled proliferation of activated lymphocytes and macrophages and could present as ALF, particularly during infancy. HLH could be inherited or acquired following infection due to over activation of natural killer cells and of CD8+ T cell lymphocytes, invariably leading to clinical and hematologic alterations. It is associated with defective apoptosis and reduced cytotoxic activity. Familial HLH is an autosomal recessive disease seen mostly in infancy and early childhood. The mutations result in reduced or defective production of cytoplasmic granules such as perforin in cytotoxic cells resulting in paradoxical over activation. Familial HLH is classified into four types based on mutation analysis. Secondary HLH usually occurs after systemic infection or immunodeficiency, which can affect people at any age and may subside spontaneously. HLH presents with fever, cutaneous rash, hepatosplenomegaly, pancytopenia, and, in severe cases, ALF [26]. ALF might be the presenting feature of hematologic malignancies such as leukemia or lymphoma [27]. Usual associated features would be fever, hepatosplenomegaly, high alkaline phosphatase, high lactate dehydrogenase, and abnormalities on peripheral blood film. Bone marrow examination confirms the diagnosis.

Autoimmune Hepatitis

Autoimmune hepatitis (AIH) can present as ALF, most of these children have positive liver–kidney microsomal (LKM) antibody (type 2 AIH). Diagnosis might be difficult, as some may not show specific antibodies at initial presentation. ALF due to AIH without encephalopathy could be benefited by immunosuppression, while ALF along with encephalopathy do not respond to any form of immunosuppression and need urgent liver transplant [28]. In our experience, of the six AIH children presenting with ALF and encephalopathy, four required liver transplantation, one died while awaiting transplantation, and one recovered with steroids [29]. The steroid responder was antinuclear/smooth muscle antibody positive (type 1 AIH), while the rest were LKM positive (type 2 AIH).

Other Causes

In spite of extensive investigation, the diagnosis could not be found in many children (indeterminate). Indeterminate ALF is probably due to unidentified infectious agent as suggested by presentation with severe hepatitis, liver failure, and bone marrow failure mimicking viral-induced disease or presentation with minimal jaundice and centrilobular necrosis on histology suggesting drug induced. More recent publications hint toward immune dysregulation and an exaggerated response of T cells causing hepatocyte damage but the exact pathways and trigger factors to be eluded [30]. There is wide variation in reporting of indeterminate etiology among various centers. This is probably due to incomplete investigations in ALF, which has been highlighted by Narkewicz et al., and labeling them as indeterminate etiology [31]. Hypoxia, bacterial infections, underlying cardiac problem, and venous outflow obstruction are few other causes of ALF.

Investigations

The etiology of ALF is so diverse that it is practically impossible to do all the tests during initial evaluation. A detailed clinical history and thorough general examination could give valuable clue of underlying problem and could help in directing management while awaiting confirmatory results. The first-line investigations should include complete blood count, liver function tests, serum electrolytes, uric acid, lactate, cholesterol/triglyceride, amylase, coagulation studies (INR), and blood glucose. Surveillance blood and urine cultures should be collected prior to starting antibiotics. The clinical presentation along with the results of first-line investigations will guide further specialized tests. Investigations to establish etiological diagnosis are outlined in Table 73.1. Supportive management along with anticipatory manage-

Table 73.1 Disease-specific investigations in acute liver failure

Infective
Serologic/quantitative tests
Hepatitis A: Anti-HAV IgM antibody
Hepatitis B: HBsAg, HBcAb(IgM), HBcAg
Hepatitis C: Anti-hep C antibody, hep C PCR
Hepatitis D: Anti-hep D antibody
Hepatitis E: Anti-HEV antibody(IgM)
Human immunodeficiency virus (HIV)
Herpes simplex virus (neonates)
Cytomegalovirus, Epstein–Barr virus
If indicated: Measles/varicella/adenovirus/echovirus/leptospirosis/
Cultures
Bacterial cultures: Blood, urine, stool, throat swab, sputum
Skin lesion if present, ascitic fluid if present
Viral culture of urine and skin lesion if present
Metabolic
Galactosemia: Galactose-1-phosphate uridyl transferase
Tyrosinemia: Urinary succinylacetone
Fructose intolerance: Quantitative enzyme assay, q22.3 band mutation in chr 9
Mitochondrial disorders: Quantitative mitochondrial DNA assay, mutation analysis
Congenital disorders of glycosylation: Transferrin isoelectrophoresis
MCAD deficiency: Plasma acylcarnitine
Wilson's disease: Serum copper and ceruloplasmin
24-h urinary copper pre- and post penicillamine
Type 1 and 2 autoimmune hepatitis
Immunoglobulins
Antinuclear antibodies
Smooth muscle antibody
Liver cytosol antibodies
Soluble liver antigen
Liver–kidney microsomal antibody
Antineutrophil cytoplasmic antibodies
Hematological malignancy
Bone marrow examination
Ascitic or cerebrospinal fluid cytospin
Genetics for HLH
Drugs and toxins: Drug levels in serum/urine
Budd–Chiari syndrome: Ultrasound, echocardiography, computed tomography
Neonatal hemochromatosis: Lip biopsy

HAV hepatitis A virus, *HEV* hepatitis E virus, *HBcAg* hepatitis B core antigen, *HBsAg* hepatitis B surface antigen, *HLH* hemophagocytic lymphohistiocytosis, *IgM* immunoglobulin M

ment of possible complications associated with liver failure would help in favorable outcome.

Prognosis

Categorical demarcation between spontaneous liver recovery and irreversible ALF is difficult. In adults with non-acetaminophen-induced ALF, King's College Hospital criteria (KCHC; Table 73.2) is used for prognostication and the need for liver transplantation. Fulfillment of KCHC is usually associated with death unless transplanted [32]. But in children, KCHC does not reliably predict death with a poor positive predictive value of 33% [33]. Of the several prognostic markers that has been proposed to predict outcomes in ALF in children, INR and factor V concentration remains the best indicators. In children with ALF, INR 4, bilirubin 235 μmol/L, age < 2 years, and WBC > 9×10^9/L are associated with poor outcome without liver transplantation [34]. Bhaduri and Mieli-Vergani have shown that the maximum INR reached during the course of illness was the most sensitive predictor of the outcome, with 73% of children with an INR less than 4 surviving compared with only 4 of 24 (16.6%) with an INR greater than 4 [35]. French centers use factor V concentration for prognostication and a value of less than 20% of normal (Clichy criteria) suggests a poor outcome. The new Wilson index proposed by Dhawan et al. based on serum bilirubin, serum albumin, INR, aspartate aminotransferase (AST), and white cell count (WCC) at presentation identified a cutoff score of 11 for death and proved to be 93% sensitive and 98% specific, with a positive predictive value of 88% (Table 73.3) [36]. In acetaminophen overdose, metabolic acidosis with arterial pH less than 7.3, after the second day of overdose in adequately hydrated patients, is associated with 90% mortality. In acetaminophen over-

Table 73.2 King's College Hospital criteria for liver transplantation

Non-acetaminophen acute liver failure
INR greater than 6.5 or
Three of the following five criteria
Patient age <11 or >40
Serum bilirubin >300 μmol/L
Time from onset of jaundice to the development of coma >7 days
INR greater than 3.5
Drug toxicity
Acetaminophen-induced acute liver failure
Arterial pH < 7.3 (after fluid resuscitation) or
All three of the following criteria
INR >6.5
Serum creatinine >300 μmol/L
Encephalopathy (grade III or IV)

INR international normalized ratio

Table 73.3 Wilson's disease index

Bilirubin (μMol/L)	INR	AST (IU/L)	WBC (10^9/L)	Albumin (G/L)	Score
0–100	0–1.2	0–100	0–6.7	>45	0
101–150	1.3–1.6	101–150	6.8–8.3	34–44	1
151–200	1.7–1.9	151–200	8.4–10.3	25–33	2
201–300	2.0–2.4	201–300	10.4–15.3	21–24	3
>300	>2.5	>300	>15.4	0–20	4

AST aspartate transaminase, *INR* international normalized ratio, *WBC* white blood cell count

dose, KCHC could be used in children for selecting candidates requiring liver transplantation (Table 73.2).

Table 73.3 showing Wilson's disease index, which has five parameters, and a score of 11 or more indicates the need for liver transplant.

Management

Due to unpredictable course of liver failure, management has to be carried out alongside of investigation. Early liaison and transfer to a specialist center with transplantation facilities is crucial for better outcome.

General Measures

Children with ALF should be monitored in a quiet setting. Vital parameters, such as pulse, blood pressure, oxygen saturation, and neurologic observations, should be done on regular basis. Prophylactic broad-spectrum antibiotics and antifungals should be started in all children, and acyclovir should be added in infants and neonates. Children with encephalopathy or an INR greater than four should be admitted to an intensive care unit for close monitoring. Hypoglycemia should be avoided by use of intravenous glucose infusion or by ensuring adequate enteral intake. The idea of protein restriction to limit the possibility of HE has now been disregarded and adequate calories should be provided. A plant protein-based diet which has more of branched-chain amino acid (BCAA) is preferred over animal protein which has more of aromatic amino acid (AAA). Oral or nasogastric feeding is usually well tolerated. Prophylactic histamine 2 blockers or proton pump inhibitors should be started to all patients requiring mechanical ventilation as stress ulcers can cause bleed [37].

N-Acetylcysteine (NAC)

N-acetylcysteine (NAC) is being increasingly used in non-acetaminophen-induced ALF as it enhances circulation and improves oxygen delivery. Retrospective study from King's College Hospital has shown that NAC infusion in non-acetaminophen-induced ALF was associated with a shorter length of hospital stay, higher incidence of native liver recovery without transplantation, and better survival after transplantation [38]. In a prospective, double-blind trial in adults with non-acetaminophen ALF, NAC usage is associated with significant improvement in transplant-free survival in patients with early (stage I–II) coma, indicating the necessity for early initiation of treatment [39]. But a more recent prospective study on NAC usage in children with non-acetaminophen ALF failed to show any significant benefit when compared to placebo [40].

Airway and Ventilation

Elective intubation and mechanical ventilation should be considered in patients with grade 1 or 2 encephalopathy that are agitated or planned to transfer and in all patients with grade 3/4 encephalopathy. Apart from providing secure airway, mechanical ventilation helps in reducing sudden variation of intracranial pressure (ICP). Sedation could be maintained with a combination of an opiate such as morphine or fentanyl and a hypnotic such as midazolam. Peak end-expiratory pressure above 8 cm of water should be avoided because it may increase ICP.

Fluid Management and Renal Failure

Intravenous fluids should be restricted to two thirds maintenance, with the idea of decreasing the possibility of development of cerebral edema. Ultrasonic cardiac output monitor (USCOM), which is a noninvasive method to measure cardiac parameters, helps in decision-making regarding appropriate fluid regimens/inotropes even in small infants. In ALF, there would be hyperdynamic circulation with decreased systemic vascular resistance, and in the presence of persistent hypotension, first-line inotropic agent of choice would be noradrenaline followed by vasopressin analogues. Continuous filtration or dialysis should be considered when the urine output is less than 1 mL/kg/h to prevent acidosis and volume overload.

Neurologic Complications

Encephalopathy is not always recognizable in children and usually is a late feature. The most serious complications of ALF are cerebral edema with resultant intracranial hypertension and HE. Encephalopathy that occurs in ALF is categorized as type A, according to the suggested nomenclature by Working Party at the 11th World Congress of Gastroenterology. HE could be clinically graded from 1 to 4 using West Haven criteria (Table 73.4), and consciousness levels could be assessed using Glasgow coma scale, which has lesser interobserver variability [41] (Table 73.5).

Clinical features of raised ICP would include systemic hypertension, bradycardia, hypertonia, and hyperreflexia and in extreme cases decerebrate or decorticate posturing. Electroencephalographic (EEG) changes occur very early in HE, even before the onset of psychological or biochemical disturbances. Ammonia-lowering measures such as dietary

protein restriction, bowel decontamination, or lactulose are of limited or no value in rapidly advancing encephalopathy. Mannitol is an osmotic diuretic commonly used to treat intracranial hypertension. A rapid bolus of 0.5 g/kg as a 20% solution over a 15-min period is recommended, and the dose can be repeated if the serum osmolarity is less than 320 mOsm/L. In ventilated patients, prophylactic hyperventilation provides no role as hypocapnia could decrease cerebral perfusion and $PaCO_2$ should be kept between 4 and 4.5 kPa. In case of clinical features of acute rise in ICP, hyperventilation could be done for a brief period of time until there is symptomatic improvement. Invasive ICP monitoring using special catheters helps in objective measurement of ICP. This helps to maintain optimal cerebral perfusion pressure (mean arterial blood pressure—ICP) of more than 50 mm Hg. Inotropic agents could be used to increase mean arterial blood pressure to achieve the optimal cerebral perfusion pressure. Studies have shown sodium thiopental, mild cerebral hypothermia (32–35 °C), and hypernatremia (serum sodium >145 mmol/L) improve cerebral perfusion. Table 73.6 shows some of the pathophysiological changes that lead on to multiorgan dysfunction associated with ALF.

Table 73.4 West Haven criteria for grading of mental state

Grade 0	Normal
Grade 1	Euphoria or anxiety
	Shortened attention span
	Impaired performance of addition
	Trivial lack of awareness
	Inverted sleep pattern
Grade 2	Subtle personality change
	Minimal disorientation for time or place
	Impaired performance of subtraction
	Lethargy or slow response
	Tremor and hypoactive reflexes
Grade 3	Somnolence to semi stupor, but responds to verbal stimuli
	Confusion, gross disorientation
	Inappropriate behavior
	Brisk reflexes and Babinski's sign
	Muscle rigidity
Grade 4	Deep coma (unresponsive to verbal or noxious stimuli)

Table 73.5 Glasgow coma scale

	Infants	Children	Score
Eye opening	Open spontaneously	Open spontaneously	4
	Open in response to verbal stimuli	Open in response to verbal stimuli	3
	Open in response to pain only	Open in response to pain only	2
	No response	No response	1
Verbal response	Coos and babbles	Oriented, appropriate	5
	Irritable cries	Confused	4
	Cries in response to pain	Inappropriate words	3
	Moans in response to pain	Incomprehensible words or nonspecific sounds	2
	No response	No response	1
Motor response	Moves spontaneously and purposefully	Obeys commands	6
	Withdraws to touch	Localizes painful stimulus	5
	Withdraws in response to pain	Withdraws in response to pain	4
	Responds to pain with decorticate posturing (abnormal flexion)	Responds to pain with flexion	3
	Responds to pain with decerebrate posturing (abnormal extension)	Responds to pain with extension	2
	No response	No response	1

Coagulopathy

As INR is a dynamic indicator of disease progression, coagulopathy should be corrected only if the patient is having a bleed or prior to an invasive procedure. Bleeding manifestation is very unusual in ALF as there would be proportional reduction in plasma levels of both procoagulant and antico-

Table 73.6 Pathophysiology of complications in ALF

Coagulopathy
Decreased levels of coagulation factors, decreased protein C, protein S, and antithrombin associated with dysfunctional platelets and fibrinogen
Encephalopathy and intracranial hypertension
Inhibitory effect of ammonia and gamma-aminobutyric acid on neuronal cell membranes and synapses. The direct toxicity of toxins on neuronal cells and vasogenic imbalance leading to intracellular fluid shifts resulting in cerebral edema
Renal failure
Acute tubular necrosis secondary to complications of ALF such as sepsis, bleeding, and/or hypotension
Hepatorenal syndrome because of renal vasoconstriction probably due to release of vasoactive mediators
Hypotension
Decreased systemic vascular resistance and hypovolemia secondary to shift of fluids into interstitial space. Adrenal insufficiency *due to* decreased liver synthesis of apolipoprotein A-1, the major protein component of HDL leading to decreased HDL and thereby decreased cortisol production
Metabolic derangement
Hypoglycemia due to increased plasma insulin levels owing to reduced hepatic uptake, reduced glycogen stores, and impaired gluconeogenesis. Lactic acidosis is related to inadequate tissue perfusion due to hypotension and also due to decreased detoxification by the liver
Infection
Impaired Kupffer cell and polymorphonuclear function along with reduced levels of factors such as fibronectin, opsonins, chemo attractants, and components of the complement system

HDL high-density lipoprotein

agulant proteins [42]. Thromboelastography would be an appropriate tool in diagnosing risk of bleeding. To correct coagulopathy, fresh frozen plasma could be given at a dose of 10 ml/kg and cryoprecipitate at 5 ml/kg (if fibrinogen is <1 g/l). In resistant cases, factor VII concentrates improve the coagulopathy for a short period. Platelet count should be maintained above 50 × 10^9/dL, as thrombocytopenia is an important risk factor for hemorrhage.

Disease-Specific Management

Antioxidant cocktail (*NAC*, *selenium*, *desferrioxamine*, *prostaglandin E1*, *vitamin E*) has been used in NH, based on the concept that iron chelation and free radical scavenging would prevent liver damage, with no proven benefit. As GALD is the common cause of NH, evidence is accumulating toward the usefulness of high-dose intravenous immunoglobulin (IVIG; 1 g/kg), in combination with exchange transfusion resulting in significant decrease in the need for liver transplantation in NH [43]. IVIG when given as prophylaxis to mothers whose previous pregnancy/child was affected with NH at a dose of 1 g/kg bodyweight, weekly from the 18th week until the end of gestation, has been associated with milder phenotypic expression of the disease and 100% survival of babies [44]. The management of HLH consists of liver supportive therapy, chemotherapy, and hematopoietic stem cell transplantation (for genetically verified/ familial disease and persistent/reactivation of secondary disease) [26]. Dietary intervention with restriction of phenylalanine and tyrosine together with oral medication, 2(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), helps in normalization of liver function in tyrosinemia type 1 but does not prevent long-term risk for development of hepatocellular carcinoma [45]. In *Amanita phalloides* (death cap) poisoning, benzylpenicillin given at a dose of 1,000,000 U/kg/d followed by 500,000 IU/kg for the next 2 days prevents toxin uptake by liver cells. In all the abovementioned conditions (excluding hematological malignancy), liver transplantation has to be considered in case of failed medical management. The beneficial role of corticosteroids in indeterminate ALF has not yet been proven in prospective studies [46].

Liver Assist Devices

Release of toxins and inflammatory mediators from the necrosed hepatocytes initiates cascade of events which could ultimately lead to multiorgan failure, and this elicits a vicious cycle of autointoxication (Fig. 73.3). Liver assist devices are developed to interrupt the cycle of autointoxication by removing toxins there by providing an opportunity for the liver to regenerate. An ideal extracorporeal liver support system has to perform complex synthetic, detoxifying, and biotransformatory functions of hepatocytes and Kupffer cells. Creating such a perfect device with possibility of providing an extracorporeal liver support system for short periods while the native liver regenerates or a liver transplant becomes available is still experimental. Currently available liver support devices could be broadly classified into cell-free cleansing devices and bioartificial liver support system, which contains human or animal liver cells. Cleansing devices perform only the detoxifying function of the liver, whereas bioartificial liver support systems have a theoretic advantage of providing the synthetic and detoxifying properties. These devices have shown to decrease the toxins (ammonia, bilirubin, cytokines, etc.) but have no effect on mortality. Successful use of these devises in ALF as a bridge, supporting liver function while the native liver regenerates, still remains elusive and not recommended outside research setting.

Liver Transplantation

Liver transplant remains the only proven treatment that has improved the outcome of ALF. Improved surgical techniques such as split liver grafts, reduced grafts, and living related donors have increased the timely availability of donor organs. The donor organs are usually blood group matched. In emergency situations ABO-incompatible liver transplantation could be done but is associated with lower graft survival. Careful patient selection is essential, as it would minimize patients from being listed for liver transplantation and then being removed due to spontaneous recovery. Patients, who were removed from the list due to spontaneous recovery, could have potentially ended up with liver transplantation if the organ becomes available before overt clinical improvement is appreciated. Absolute contraindications for liver transplant are fixed and dilated pupils, uncontrolled sepsis, systemic mitochondrial/metabolic disorders, and severe respiratory failure [47, 48]. Relative contraindications are increasing inotropic requirements, infection under treatment, cerebral perfusion pressure of less than 40 mmHg for more than 2 h, and a history of progressive or severe neurologic problems.

Auxiliary Liver Transplantation

Auxiliary liver transplant is a surgical procedure where a healthy donor liver graft is implanted with native liver in situ. Auxiliary liver transplant could be orthotopic (part of the native liver is resected and replaced with a reduced-size graft) or heterotopic (the donor graft is placed alongside the

native liver in the right upper quadrant). Due to higher incidence of complications associated with heterotopic auxiliary liver transplant, it is no more used in clinical practice. Now the standard surgical technique is auxiliary partial orthotopic liver transplantation (APOLT). The rationale behind the use of this technique in ALF is that the allograft supports liver function while the native liver regenerates and then immunosuppression could be weaned and eventually stopped. It has been shown that complete native liver recovery could happen even when there is more than 90% hepatocyte loss [49, 50]. In a series from King's College Hospital, of the 20 children who received auxiliary liver transplantation for ALF, 10-year patient survival was 85% [51]. Among the 17 survivors, 14 (82%) have successfully regenerated their native liver of which immunosuppression was withdrawn successfully in 11 patients at a median time of 23 months after transplantation. This would be an ideal option in ALF due to indeterminate etiology, as spontaneous regeneration of native liver remains a possibility. Caution should be excised while selecting patients with hemodynamic instability and cerebral edema as deterioration might happen during postoperative period due "toxic necrotic liver" left behind.

Hepatocyte Transplantation

Hepatocyte transplantation is a procedure where hepatocytes are infused intraportally into the patient's liver, where a proportion of cells will engraft and would support liver function. This technique has been tried with variable success in certain liver-based metabolic disorders. Its use in ALF still remains experimental. Lack of surrogate markers of rejection poses an important problem, as it is difficult to titrate immunosuppression. Dhawan et al. has recently published the utility of alginate-encapsulated hepatocytes in management of ALF [52]. Alginate-encapsulated hepatocytes were injected intraperitoneally in a cohort of eight children with ALF and has found to avert LT in four and acted as bridge in three. This technique avoids the complications of coagulopathy and portosystemic shunting of infused cells and protects the cells from direct immune attack.

Conclusions

ALF in children is a medical emergency as it leads to multiorgan failure and has to be approached systematically. Newer definition for ALF obviates the need for encephalopathy to make the diagnosis. Expert intensive care facility is essential to stabilize and make these children fit for transplantation. Etiological diagnosis aids in disease-specific management. Acyclovir should be started in all neonates with ALF until results of herpes virology are available. Liver transplantation is the only definitive treatment that improves survival in pediatric ALF, while liver assist devices and hepatocyte transplantation are potential emerging therapies.

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Complications of Cirrhosis in Children

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Introduction

Cirrhosis is the end result of progressive fibrosis irrespective of the insult to the parenchyma or biliary tree. Unlike adults, there is no time duration of illness that defines chronic liver disease (CLD). All the inherited metabolic and genetic conditions even when diagnosed at birth could have advanced fibrosis or cirrhosis. Cirrhosis is a histological diagnosis but is liberally used in clinical practice without histological evidence. Liver cell death leads to a cascade of immunological reactions leading to activation of stellate cells with subsequent excessive collagen production and ultimately fibrosis. Bridging of the portal tracts (porto-portal) or the central vein to the portal tracts with nodule formation and disruption of the liver architecture is defined as cirrhosis. The disruption of the liver architecture leads to the disturbance in hemodynamics of blood flow into the liver and homeostasis of synthetic and detoxification functions of the liver, resulting in complications such as portal hypertension (PHT), ascites, and encephalopathy. This can lead to a secondary cascade of events affecting the function of several end organs such as the brain, kidney, and lungs [1]. Understanding the pathophysiology of the complications in CLD helps in anticipation and initiation of treatment at appropriate time. This chapter deals with the common complication associated with CLD.

Cirrhosis

The hallmark of CLD is fibrosis of the liver. Cirrhosis is a histopathological term used to describe microscopic and/or macroscopic changes in the liver characterized by aberrant nodule formation, vascular changes, and totally disturbed architecture associated with fibrosis. The degree to which the abovementioned changes progress depends on the nature and duration of the insult. Cirrhosis/fibrosis leads to complications such as PHT, ascites, encephalopathy, hepatorenal syndrome (HRS), hepatopulmonary syndrome (HPS), and eventually resulting in end-stage liver disease.

Etiology of Cirrhosis

Common diseases that cause chronic injury to the liver in children leading to cirrhosis are outlined in Table 74.1. Cell death or injury, as in hepatotropic viral infections or accumulation of toxic by-products such as hydrophobic bile acids in hepatocytes, is associated with release of several proinflammatory mediators (e.g., tumor necrosis factor, TNF) that lead to progressive fibrosis and eventually liver cirrhosis. As with any other multifactorial disease, both genetic and environmental factors play a role in cirrhosis. The pattern and type of proinflammatory mediator released depend on the underlying etiology of hepatocyte death [2]. These mediators act on Kupffer cells, hepatocytes, and cholangiocytes that release secondary mediators, which then activate hepatic stellate cells (HSC) to produce extracellular matrix (ECM). Thus, etiology of liver insult influences the pattern of fibrosis, as fibrosis is first observed in periportal area in viral hepatitis, perivenular region in toxic damage, while in developmental malformations such as biliary atresia fibrosis starts around the bile duct. Liver fibrosis is considered to be a wound-healing process, similar to any injured tissue in body, but when the response is exaggerated and persistent, it progresses to cirrhosis.

Pathogenesis of Liver Fibrosis

Persistent insult to either hepatocytes or cholangiocytes acts as a stimulus for fibrosis to set in. Hepatotropic viruses, toxins, and diseases that cause chronic cholestasis (tyrosinemia,

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Table 74.1 Some of the common causes of liver cirrhosis in children

Etiology of liver cirrhosis
Metabolic liver disease
α1-antitrypsin deficiency
Wilson disease
Progressive familial intrahepatic cholestasis
Glycogen storage diseases
(especially types IV, VI, IX, and X)
Lipid abnormalities (e.g., Gaucher disease)
Peroxisomal disorders (e.g., Zellweger syndrome)
Tyrosinemia
Cystic fibrosis
Developmental/genetic
Biliary atresia
Congenital hepatic fibrosis
Alagille syndrome
Infections
Viral (e.g., chronic hepatitis B or C)
Parasitic (e.g., echinococcosis)
Immune related
Autoimmune hepatitis
Sclerosing cholangitis
Vascular
Budd–Chiari syndrome
Hepatic veno-occlusive disease
Portal vein thrombosis
Miscellaneous
Nonalcoholic steatohepatitis (NASH)
Drugs

progressive familial intrahepatic cholestasis, etc.) damage liver cells, either by apoptosis or necrosis leading to activation of a cascade of events causing fibrosis/cirrhosis (Fig. 74.1). The damaged liver cells secrete inflammatory mediators such as transforming growth factor (TGF)-beta1, TNF-α, epidermal growth factor (EGF), insulin-like growth factor (IGF), endothelin (ET), and platelet-derived growth factor (PDGF) that activate HSC. These damaged hepatocytes/cholangiocytes also recruit and activate T cells and Kupffer cells which secrete interleukin 6 (IL-6), interferon (IFN)-alpha, CD40, CCL21, and IGF which in turn activates HSC via a different pathway. The ECM such as type IV collagen, fibrinogen, and urokinase-type plasminogen activator that is secreted during the process of fibrosis could act as a stimulus for further activation of HSC starting a vicious cycle [3].

Under normal condition, HSC are quiescent cells that store lipids and vitamin A in the liver that resides in the space of Disse. When activated, HSC transdifferentiate to myofibroblast-like cells, acquiring fibrogenic and secretory properties. Apart from HSC, myofibroblasts that are derived from small portal blood vessels and bone marrow play a role in ECM formation during fibrosis of the liver [4–6]. The source of fibroblast that is involved in fibrosis of the liver depends on the etiology of insult to the liver [6]. With biliary pathology such as biliary atresia, portal tract fibroblasts, and in hypoxic insults, bone marrow fibroblasts play an important role [7, 8].

The ECM laid during this process of fibrosis differs in structure and composition, when compared to the ECM laid during routine remodeling. Mitogenic stimuli such as TGF-beta upregulate several precollagenous genes and increase the secretion of collagen by myofibroblasts, particularly types 1 and 3. Activated HSC also enhance the secretion of ECM-digesting enzymes like interstitial collagenase, gelatinase A, and stromelysin-1 which degrade specific components of ECM such as type 4 collagen and laminin, paving way for increased composition of type 1 and 3 collagen in fibrosis [9]. Increased tissue inhibitors of matrix metalloproteinases (TIMPs), which inhibit collagenase, result in gross imbalance between production and degradation of ECM. The fibrous tissue thus formed could interconnect portal tracts, portal tracts and central venules, or a mixture of both patterns and progress to cirrhosis. Contrary to the initial belief, it is shown that liver fibrosis even cirrhosis could regress to some extent on successful treatment of underlying cause [10].

Diagnosis of Liver Fibrosis

Fibrosis of the liver covers an entire spectrum of microscopically detectable fibrosis during initial stages to macroscopically gross fibrosis as in advanced stages of CLD. There is no validated biochemical test or scoring system based on biochemical tests that would grade the severity of fibrosis [11]. Synthetic liver function or cholestasis has no correlation with the degree of fibrosis, as these parameters may be normal even in advanced fibrosis as in congenital hepatic fibrosis. Imaging techniques such as ultrasonography, computed tomography, and magnetic resonance imaging (MRI) can identify liver fibrosis though quantification is difficult. Liver biopsy is considered to be the gold standard method for the assessment of liver fibrosis as it provides necroinflammatory and the fibrosis grade. Figure 74.2a shows complete nodule formation, and Fig. 74.2b shows extensive collagen, when stained by trichrome in a cirrhotic liver. Scoring of degree of fibrosis could be done using Metavir (stages I–IV) and Ishak score (stages I–V). Due to invasive nature of the procedure, it is practically difficult to monitor fibrosis progression using liver biopsy. To overcome this problem, transient elastography (FibroScan®), a noninvasive technique, is used in serial monitoring of progress of fibrosis with good accuracy and has been validated even in children [12]. Spleen stiffness measurement using transient elastography has shown to be helpful in predicting clinically significant varices [114].

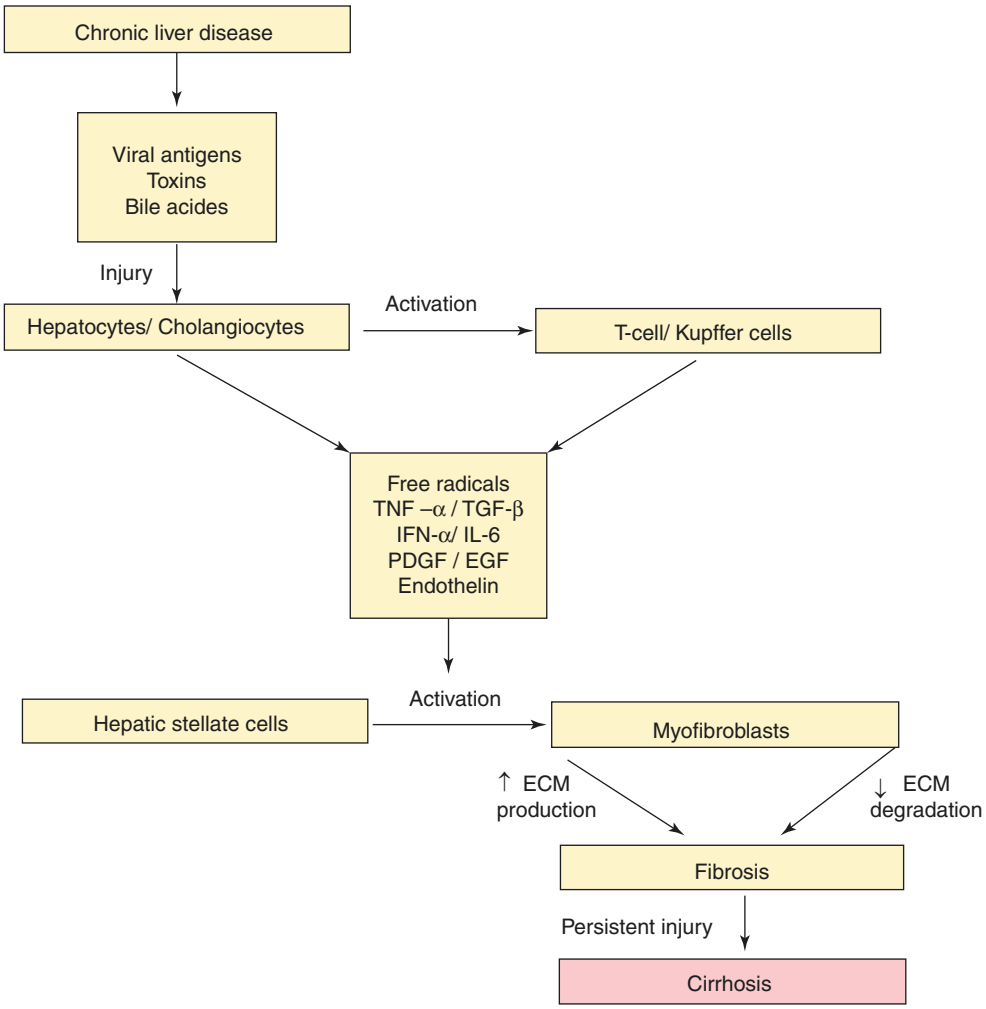


Fig. 74.1 Diagram showing cascade of events leading to liver cirrhosis

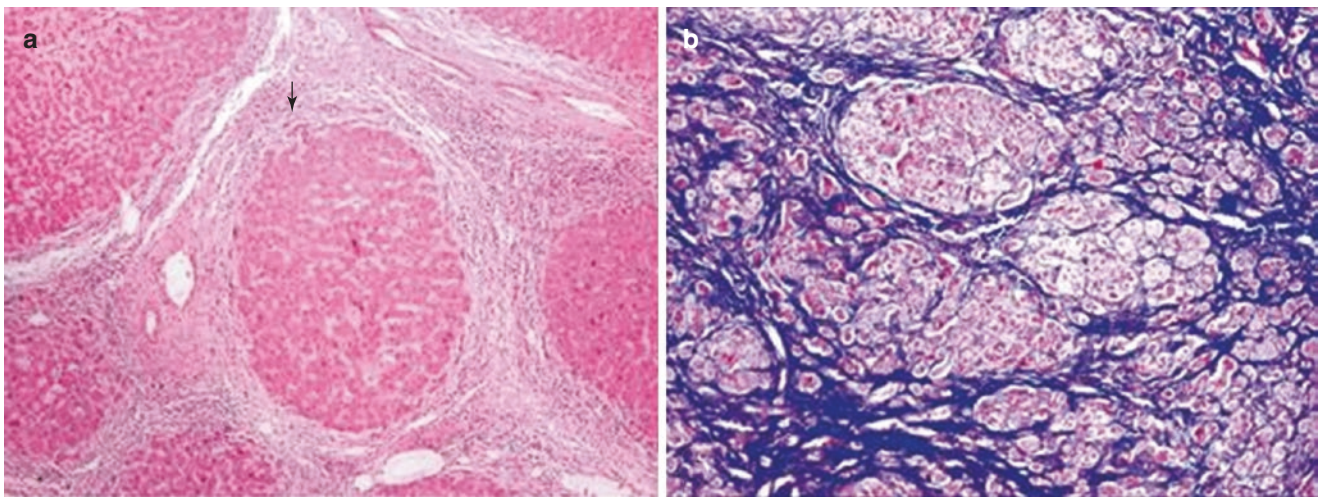


Fig. 74.2 (**a**) Microscopic image of cirrhotic liver showing a nodular formation. Hepatic lobule (*arrow head*) completely encased by fibrous tissue. (**b**) Microscopic image of trichrome-stained liver, where collagen is stained *blue*, while the cytoplasm of hepatocytes is stained *red* and nuclei as black structures within cells

Complications and Management of Cirrhosis

CLD and the term "cirrhosis" have been used interchangeably in literature as cirrhosis is a manifestation of CLD, and most of the complications in CLD are secondary to cirrhosis. With progressive fibrosis, there is distortion of blood vessels and bile leading to increased resistance to blood flow resulting in increased portal pressure on the afferent side and tissue hypoxia due to reduced blood supply on the efferent side. Cirrhosis also leads to increase in hepatic artery resistance, and in the case of hypotension secondary to an infection or variceal bleed, the liver could suffer hypoxic insult and could decompensate. Over a period of time, the fibrous tissue coalesces to form a tight capsule and restricts hepatocyte regeneration and worsens cholestasis. Synthetic/detoxification failure, cholestasis, and fibrosis individually or in combination contribute toward complication of CLD, some of which are highlighted in Table 74.2. There is an increased risk of hepatocellular carcinoma (HCC) in cirrhotic liver, and the generalized nodular transformation of the liver makes it difficult to diagnose HCC at early stages. Ultimately, these patients would succumb either to liver failure or to its complication unless treated appropriately. Fibrosis is a reversible phenomenon, but the extent to which the distorted liver architecture that would revert back to its original state still remains an unanswered question. Disease-specific treatment, for example, chelation for Wilson's disease and steroids for autoimmune liver diseases, halts the progress of fibrosis and could reverse cirrhosis. At present, there is no approved drug that would inhibit or reverse fibrosis.

Table 74.2 Signs and symptoms of cirrhosis and probable mechanism causing it

Complications of cirrhosis		
Symptoms	Signs	Underlying mechanism[a]
Jaundice, pruritus	Elevated bilirubin and bile acids	Detoxification failure of liver
Altered mental status	Encephalopathy	Detoxification failure of liver
Distended abdominal veins, vomiting blood	Caput medusae	Portal hypertension
Abdominal distension swelling of legs	Ascites/edema	Hypoalbuminemia, portal hypertension
Muscle wasting	Malnutrition	Decreased absorption of nutrients due to defective bile flow and increased demand
Telangiectasia	Spider nevi	Detoxification failure of certain hormones
Easy fractures	Osteoporosis and osteomalacia	Hepatic osteodystrophy
Decreased urine output	Oliguria	Hepatorenal syndrome
Bluish discoloration and swelling of fingers	Cyanosis and clubbing	Hepatopulmonary syndrome, portopulmonary hypertension
Difficulty in breathing	Dyspnea	Hepatopulmonary syndrome, portopulmonary hypertension

[a]More than one mechanism could be involved

Complications of Cirrhosis

Portal Hypertension

The liver is a unique organ with dual blood supply, via the hepatic artery and portal vein. The portal vein, formed by the splenic vein and superior mesenteric vein, supplies nutrient-rich blood from the intestine to the liver. The usual pressure is less than 5 mmHg, and this blood traverses hepatic sinusoids reaching hepatic venules having much lower pressures and enters systemic venous circulation. PHT develops when there is increased resistance anywhere along the portal system, and thus PHT could be extrahepatic (portal vein thrombosis), intrahepatic (cirrhosis), or posthepatic (hepatic venous thrombosis). PHT leads to opening up of portosystemic collaterals causing varices, which could rupture and cause bleed.

Direct measurement of portal pressure is difficult, and so hepatic venous pressure gradient (HVPG) is measured via hepatic venous catheterization. HVPG is the pressure difference between wedged hepatic venous pressure (WHVP) and free hepatic venous pressure, which is reflective of portal pressure. HVPG of more than 10 mmHg is associated with the development of varices, and pressures more than 12 mmHg is associated with variceal bleed. In clinical practice, accurate measurement of portal pressure by invasive methods is of little relevance as esophageal varices and splenomegaly hint toward the development of PHT, and this needs to be managed appropriately.

Pathogenesis of PHT in Cirrhosis

Like any other tubular organ, resistance and flow govern the pressure in the portal venous system. In CLD, there is a substantial increase in both flow and resistance of portal vein leading to net increase in portal pressure.

Increased Resistance to Portal Flow

In cirrhosis, dynamic contractile elements within sinusoids and portal venules contribute up to 40% of increased vascular resistance, while the rest is attributed to fibrosis. Wrapping of HSC and myofibroblasts can wrap around sinusoids, and

the portal venular smooth muscle contraction can increase vascular resistance [13]. ETs are a group of peptides, which cause vasoconstriction and stimulation of cell proliferation in tissue. ET-1 and ET-3 are found to be increased in cirrhotics when compared to controls, probably due to increased production [14]. Angiotensin II, norepinephrine, and thromboxane A2 (TXA2) are other vasopressors which are found to be increased in cirrhosis of the liver and cause increased vascular resistance of portal venules [15]. There is an associated endothelial dysfunction, which further increases the vascular resistance instead of decreasing it. This is probably due to increased levels of TXA2 impairing the response to endothelium-dependent vasodilator acetylcholine, and thus the resultant increased vascular resistance is probably due to imbalance between increased vasoconstrictors and decreased vasodilators in CLD [16].

Increased Portal Circulation

Splanchnic vasodilatation combined with hyperdynamic circulation contributes to increased portal circulation. Nitric oxide (NO) [17] increases the production of cyclic guanosine monophosphate, which directly relaxes the smooth muscle. Increased NO due to enhanced endothelial NO synthase (eNOS) activity in splanchnic circulation is suggested to play an important role in splanchnic vasodilatation. Increased levels of glucagon found in cirrhosis cause direct vascular smooth muscle relaxation and decrease the sensitivity of vascular smooth muscle to endogenous vasoconstrictors. Other vasodilators such as capsaicin–calcitonin gene-related peptide, neuropeptides, and adenosine have a doubtful role. Plasma volume expansion due to sodium retention in cirrhosis also plays a role in increased portal flow.

Variceal Bleed in Portal Hypertension

Apart from ascites and hypersplenism, the major complication of PHT is variceal bleed. HVPG of more than 10 mmHg results in opening up of portosystemic collaterals, which are situated at the lower end of the esophagus, rectum, and paraumbilical and retroperitoneal regions. Life-threatening bleeds are usually from esophageal and gastric varices that arise from a collateral network through the coronary and short gastric veins draining into the systemic azygos vein. Miga et al. showed that 20% of children had esophageal variceal hemorrhage (EVH) within 2 years after Kasai portoenterostomy, and the risk of death or need for liver transplantation was 50% at 6 years after the initial episode of EVH in a cohort of children with biliary atresia [18]. Etiology plays an important role in the survival outcome after variceal bleed, with varices due to portal vein thrombosis having better prognosis when compared with those due to intrinsic hepatobiliary disease [19].

Management of Variceal Bleed

Variceal bleed is a life-threatening emergency, and the patient needs to be stabilized before shifting for emergency endoscopy. In the event of hypovolemic shock, fluid resuscitation has to be initiated while awaiting whole blood, and in addition, fresh frozen plasma/platelets/cryoprecipitate/factor VIIa might be required, as these patients would be coagulopathic secondary to liver disease. Nasogastric tube placement and gastric lavage help to quantify the blood loss as well as to remove blood from the stomach that could precipitate encephalopathy. Vasoactive drugs that decrease portal pressure (Table 74.3) should be started along with proton pump inhibitors/H2 blockers and antibiotics. Once stable, endoscopic variceal ligation (EVL) or endoscopic sclerotherapy could be done [20]. In the case of continuous bleed and hemodynamic instability, balloon tamponade with a Minnesota or Sengstaken–Blakemore tube should be attempted. Both these tubes have esophageal and gastric balloon, but Minnesota tube has esophageal aspiration port in addition to gastric aspiration port while Sengstaken–Blakemore tube has only gastric aspiration port. These tubes need to be placed by an experienced person as improper placement or overinflation of the balloon can result in esoph-

Table 74.3 Various drugs used in portal hypertension and its mechanism of action

Medications used in portal hypertension		
Drugs used in acute variceal bleed		
Drug	Dosage	Mechanism of action
Vasopressin	Initial IV bolus 0.3 U/kg (maximum: 20 U) over 20 min followed by infusion: 0.002–0.01 U/kg/min	Acts via V1 receptors, reduces portal blood flow, portal systemic collateral blood flow, and variceal pressure
Octreotide	1 μg/kg/h IV infusion or 2–4 μg/kg/dose/8 h s.c.	Analogue of somatostatin Reduce portal flow and pressures
Terlipressin	8–20 μg/kg of body weight given at intervals of 4–8 h	Terlipressin, a prodrug of vasopressin
Drugs used to prevent rebleed		
Propranolol Atenolol	1–5 mg/kg/day in three divided doses 1 mg/kg/day in two doses (Dose has to be adjusted to decrease heart rate by 25%)	Reduces portal hypertension by decreasing cardiac output and inducing splanchnic vasoconstriction by blocking β-1 and β-2 receptors
Carvedilol	0.3 mg/kg/day	Nonselective β-blocker with additional α-1 blocking action

IV intravenous, *s.c.* subcutaneous

ageal rupture and/or ischemia [21]. Once bleeding is controlled, endoscopic sclerotherapy (ESL) and endoscopic *variceal* ligation (*EVL*) has to be performed, as conservative management (medication and balloon tamponade) without endoscopic intervention was associated with 3.6 times higher risk of rebleed [22].

ESL is a procedure where sclerosant such as ethanolamine oleate or sodium tetradecyl sulfate is injected into a varix under direct vision, thereby obliterating the vessel. Cyanoacrylates are synthetic glues used in gastric varices that rapidly solidify on contact with water and blood. Usually, cyanoacrylates are mixed with ethanolamine oleate at 1:1 ratio to decrease the rate of solidification, as it minimizes the inadvertent adherence of catheters to the endoscope. EVL is a procedure where an elastic O-ring is applied over a small area of esophageal mucosa and submucosa, causing strangulation of the tissue (varix) caught in between the ring, eventually leading to fibrosis and obliteration. Randomized trial of endoscopic sclerotherapy versus variceal band ligation for esophageal varices showed that while both are equally effective in controlling acute bleed, EVL achieved variceal obliteration in fewer treatment sessions along with significantly lower rate of the development of portal gastropathy and rebleeding [23]. Meta-analysis suggests that pharmacotherapy coupled with either EVL or ESL showed better initial bleeding control and 5-day hemostasis but no effect on mortality [24]. Occasionally, bleeding from gastric varices and Roux en Y jejunal loop varices might be difficult to control and might require interventional measures such as transjugular intrahepatic portosystemic shunt (TIPSS), surgical shunts, and esophagogastric devascularization ± splenectomy [25]. Liver transplantation has to be offered to those with CLD, after control of the acute bleeding episode.

Prophylactic Therapy for Variceal Bleed

The American Association for the Study of Liver Diseases (AASLD) practice guidelines recommend β-blockers as first-line therapy in adults with medium/large esophageal varices and EVL for patients in whom β-blockers are contraindicated or poorly tolerated [26]. Trials on children showed that β-blockers and EVL/ESL are relatively safe to be used, but so far, there are no robust data to suggest that its use as primary prophylaxis decreases the incidence of first episode variceal bleed in children [27, 28]. Variceal eradication and nonselective beta-blocker are recommended in adults after an episode of bleed as secondary prophylaxis, while in children, small studies have shown usefulness of EVL/EST to prevent rebleed, but the role of beta-blocker is still unclear [29, 30].

Neurological Complications in Cirrhosis

CLD predisposes to a variety of neurological complications such as intracranial hemorrhage due to the presence of coagulopathy, increased risk of cerebral infection due to decreased immune function, and hepatic encephalopathy (HE) [31].

Hepatic Encephalopathy

HE is defined as a metabolically induced, potentially reversible, functional disturbance of the brain that may occur in acute or CLD [32]. Though HE is considered to be a potentially reversible condition, some patients might not recover to their previous level of cognitive function after a severe episode of HE.

Factors such as infection, trauma, and electrolyte imbalance in the background of CLD could cause sudden liver decompensation and lead to HE. Encephalopathy that occurs in acute liver failure (ALF) is categorized as type A, HE in portosystemic shunt without any intrinsic hepatocellular disease as type B, and in CLD as type C. Type C could be further subclassified into minimal, episodic, or persistent. HE is called minimal hepatic encephalopathy (MHE), when diagnosis could be made only on psychometric analysis in an apparently normal person with CLD. Episodic encephalopathy in CLD coincides with episodes of high protein intake, gastrointestinal bleed, infection, etc. Usually, these episodes resolve with the treatment of the precipitating factors, but sometimes they persist and are termed as persistent HE.

Clinical Features

HE could be clinically graded from 1 to 4 using West Haven criteria, and Glasgow coma scale which has lesser interobserver variability could be used in assessing conscious levels [33]. MHE is associated with mild intellectual impairment, where verbal ability is preserved and apparently normal personality [34]. Worsening of HE leads to asterixis (flapping tremor), hypertonia and hyperreflexia, hypotonia, and areflexia that may be seen with subsequent progression to coma. Features similar to Parkinsonism such as muscular rigidity, bradykinesia, hypokinesia, monotony of speech, and tremors could be seen in HE.

Pathogenesis of Hepatic Encephalopathy

The pathogenesis of HE is thought to be multifactorial, with neurotoxins and altered neurotransmitters acting on the neurons. Ammonia is considered to be an important factor

involved in pathogenesis, while mercaptans, short- and medium-chain fatty acids, phenols, methionine derivatives, etc. play a minor role, altering the ratio of excitatory (↓ dopamine and noradrenaline) and inhibitory (↑ gamma-aminobutyric acid (GABA) and serotonin) neurotransmitters resulting in abnormal cerebral function. Apart from these, there is an increased formation of false neurotransmitters such as octopamine, phenyl methionine from accumulation of phenylalanine, and tyrosine. There are other proposed mechanisms of HE such as an increase in false neurotransmitters (octopamine, phenyl methionine), altered ratios of branched-chain amino acids (BCAA) and aromatic amino acids (AAA), changes in postsynaptic receptor activity, increased permeability of blood–brain barrier, etc.

Role of Ammonia in HE

Ammonia is produced from a variety of sources such as the small intestine enterocytes, gut flora, muscle, and kidney. Under healthy condition, around 80–90% of ammonia is either converted to urea by periportal hepatocytes or glutamine by perivenous hepatocytes. Though ammonia is implicated in HE, the exact mechanism is still elusive. Few of the suggested mechanisms are as follows: (1) Excess ammonia is converted to glutamine by glial cells. This glutamine increases intracellular osmotic pressure, leading to swelling of astrocytes and cerebral edema, and can competitively bind to glutamate receptors and inhibit them. (2) Oxidative stress triggered by ammonia toxicity in the astrocyte resulting in mitochondrial dysfunction. (3) Enhanced cytokine activity and impaired intracellular signaling [35].

It was thought that ammonia in portal circulation was exclusively produced by the gut flora, but current hypothesis suggests that small gut enterocytes produce ammonia in excess of gut flora, and gut decontamination alone has minimal effect on ammonia levels [36]. The kidney is involved in both production and elimination of ammonia. The kidney can metabolize glutamine to produce ammonia and bicarbonate and can excrete ammonia as ammonium ion and urea in urine [35]. Alkalosis can decrease the conversion of ammonia to ammonium, as there is no need to excrete the hydrogen ion resulting in elevated free ammonia that can cross blood–brain barrier and precipitate HE. Myocytes can act as buffer by converting ammonia to nontoxic glutamine via glutamine synthetase, and thus poor muscle mass is an important risk factor for HE [37].

Role of Neurotoxins and Inflammatory Mediators in HE

The ratio of BCAA/AAA phenylalanine, tyrosine, and tryptophan is called the Fischer ratio [38, 39]. There is decrease in Fischer ratio in liver failure, due to preferential usage of BCAA by muscles and decreased clearance of AAA by liver. Elevated serum AAA can cross the blood–brain barrier into the brain and results in synthesis of false neurotransmitter such as octopamine and synephrine [40].

Several short-chain fatty acids such as propionate, butyrate, and valerate are produced in the small intestine through the breakdown of proteins by fecal flora [41]. These short-chain fatty acids competitively inhibit urea cycle enzymes and can bind to albumin and displacing albumin-bound toxins, thus precipitating HE. Indole, oxindole, endozepines, neuronal acetylcholinesterase, TNF-α, and allopregnanolone are few of the other bioactive substances that could play a role in HE [42–46].

Diagnosis

Diagnosis of encephalopathy is mainly based on clinical history and examination [47]. Clinical diagnosis of mild-to-moderate HE is difficult in children as it requires their cooperation to perform psychometric tests. Neuroimaging and electrophysiological studies of the brain would give supportive evidence rather than to confirm the diagnosis of HE. Though arterial ammonia level correlates with the severity of HE, it could not be used to grade encephalopathy [33]. HE could be clinically graded using West Haven criteria, and conscious level is graded using Glasgow coma scale [33].

Neuropsychological Assessment

In children, neuropsychological assessment should be made using tests such as Wechsler intelligence tests and Dutch child intelligence test (Revisie Amsterdamse Kinder Intelligentie test), where validated nomogram exists for the selected age, sex, racial, and ethnical subgroup. The Psychometric Hepatic Encephalopathy score (PHES) and number connect test (NCT) A and B could be used to diagnose MHE in adults while these tests are not validated in children [48, 49].

Critical Flicker Frequency

Critical flicker frequency threshold is a simple and reliable test for the quantification of low-grade HE [50]. The principle of the test is to identify the point of switch over from a steady red light to a flickering light. A normal human eye perceives flicker rate of 60 Hz as a steady light. The frequency of the light is gradually decreased to a point where the patient starts to perceive the light as flicker. Using this technique, it was found that patients with HE can pick up

flickering only when the flicker rate comes below 39 Hz, while cirrhotic counterparts without encephalopathy and normal individual could identify flickering at higher rate [47]. The need for understanding (and being able to follow instructions) the test limits is used only in children more than 8 years [51].

Electroencephalogram

Using electroencephalogram (EEG), HE could be graded into five grades (0–4). Grade 0 is a normal EEG with regular alpha rhythm. Grade 1 encephalopathy is characterized by irregular background alpha rhythm and appearance of theta rhythm. Theta activity becomes continuous with occasional delta wave in grade 2 HE, and theta activity becomes prevalent with transient polyphasic complexes of spikes and slow waves in grade 3. Grade 4 is deep coma, having continuous delta waves with abundant complexes of spikes and slow waves [47]. With the availability of newer complex EEG analytical softwares, EEG can be analyzed in specific regions of the brain.

Neuroimaging in HE

Computed tomography (CT) of the brain is not a useful tool in diagnosis of HE, apart from excluding organic causes such as bleed, tumors, or gross edema that can cause encephalopathy [52]. MRI is a better modality to diagnose cerebral edema and demyelination associated with HE. Conventional MRI lacks sensitivity in diagnosing milder forms of edema, and newer techniques such as magnetization transfer imaging (MTI), fast fluid-attenuated inversion recovery (FLAIR) imaging, and diffusion-weighted imaging are more sensitive in picking up brain tissue water content [53]. Proton MR spectroscopy could measure different levels of metabolites in the brain such as myo-inositol, choline, and glutamine. These specialized neuroimaging is more of research interest and provides little benefit in routine clinical practice.

Management of Hepatic Encephalopathy

Management should be directed toward stabilization of the patient and treating the precipitating factors such as bleeding, electrolyte imbalance, and infection. In HE of grade 3 or 4, elective intubation has to be considered to protect the airways. Despite increased total body water, these patients would be intravascularly fluid depleted. Hydration is best monitored by central venous pressure (CVP) and ideally should be 6–8 cm of H_2O. Though their serum sodium is low, they will have high total body sodium, and any attempt to normalize the sodium will lead to worsening of edema. Supplementation of extra sodium is required if the sodium level falls below 120 mEq/l [54].

Lactulose decreases the colonic pH to around 5, thereby decreasing the bacterial fermentation and production of short-chain fatty acids [55, 56]. In acidic environment, the ammonia produced in the colon is converted to ammonium ion, reducing its diffusibility back into circulation, and thereby removed along with stools. The amount of lactulose dose has to be titrated to produce three soft stools/day. Long-term lactulose in CLD helps to prevent HE but will not change the ultimate requirement of a liver transplantation. Lactitol (B galactosido-sorbitol) is a nonabsorbable sugar which has similar action to lactulose but less sweeter and more palatable [57].

Neomycin, vancomycin, and rifaximin are few of the antibiotics that are used to eliminate ammoniagenic bacteria from the gut, thereby decreasing the ammonia production in the intestine [58]. Of all the antibiotics, rifaximin has been shown to be very effective in decreasing ammonia production and has highest risk–benefit ratio. Rifaximin and lactulose given together were found to be more beneficial than either of them given alone [59]. The use of probiotics has shown to reduce plasma ammonia, but there was no change in final outcome [60].

L-ornithine-L-aspartate (LOLA) enhances the action of ornithine and aspartate transaminases in the brain and peripheral tissues to produce nontoxic glutamate. There is no standard dosing in children; up to 20 g/day could be given at an infusion rate not exceeding 5 g/h as suggested by drug monogram and not recommended in children less than 8 years old [54]. Sodium benzoate (250 mg/kg/day) along with intravenous sodium phenylacetate (250 mg/kg/day) or oral sodium phenylbutyrate (250 mg/kg/day) could be used to eliminate ammonia by alternate pathways, thereby decreasing serum ammonia levels. Water-soluble hippuric acid and phenylacetyl-glutamine are formed by conjugation of benzoate with glycine and phenyl acetate with glutamine, respectively. Hippuric acid and phenylacetyl-glutamine are then excreted by the kidneys, thereby reducing ammonia load [61]. Though all these medications are more useful in hyperammonemia due to urea cycle defects, they are used off-label in hyperammonemia due to liver failure [35].

There has been increasing interest in the possibility of providing an extracorporeal liver support system for short periods while liver allograft becomes available. High cost, nonavailability of small filters, and lack of safety data in children limit the usage of these devises to clinical trial setting. Liver transplantation has shown to be the definitive treatment, which has improved survival outcome both in adults and children. Elective liver transplantation offers 5-year survival of more than 85% in children. Synthetic liver failure with encephalopathy is one of the indications

for liver transplantation in CLD, which has to be done before permanent neurological damage sets in.

Ascites

Fluid retention and low albumin in CLD result in accumulation of fluid in extravascular space leading to edema, ascites, and plural effusion. Presence of ascites is one of the variables that increase the mortality in children with CLD [62]. Onset of ascites indicates decompensation of liver disease and the need for liver transplantation. Ascites increases the risk of bacterial peritonitis and HRS, which potentially adds on to the already increased mortality associated with liver decompensation (see Fig. 74.3).

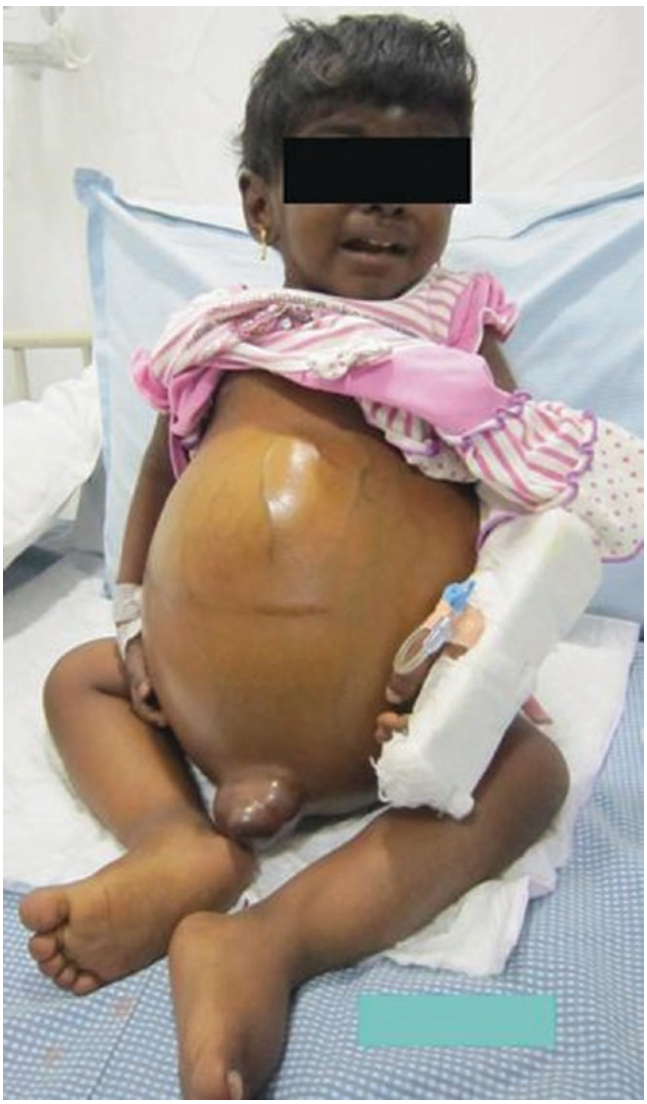


Fig. 74.3 Image of a child with failed Kasai portoenterostomy showing gross ascites, dilated abdominal veins, and edema of legs

Pathophysiology of Ascites

There are several theories based on the triggering factor of the vicious cycle that leads to fluid retention and ascites. The "overfill" theory suggests hepatorenal reflex to be the primary event that leads to sodium and fluid retention and subsequently ascites. The "underfill" theory suggests that that the increased pressure in portal sinusoids results in increased hydrostatic pressure leading to excessive lymph production. When lymph production exceeds absorption, the net result is ascites and contraction of intravascular volume (underfill). "Peripheral arterial vasodilation hypothesis" which was proposed in 1988 suggests that splanchnic and peripheral arterial vasodilatation secondary to PHT and cirrhosis is the initial triggering event [63]. This causes decreased intravascular volume, leading to baroreceptor-mediated activation of renin–angiotensin–aldosterone system and release of antidiuretic hormone, resulting in hypervolemic stage due to renal sodium and water retention [63–65]. Splanchnic vasodilatation along with hypervolemia results in increased hydrostatic pressure that ultimately results in passage of fluid to the peritoneal space [66]. It is difficult to explain the complex cascade of events leading to ascites based on one theory, and it is possible that mechanisms as suggested by different theories could contribute to various stages of ascites formation.

Biochemical Diagnosis

Ascitic tap should be carried out in any new-onset ascites. Estimating serum–ascites albumin gradient (SAAG) is considered to be superior in making differential diagnosis of probable cause of ascites, compared to quantifying it as exudate or transudate. SAAG $\geq$ 1.1 g/dL is associated with cirrhosis, portal or hepatic venous occlusion, congestive cardiac failure, etc., while SAAG < 1.1 g/dL is associated with nephrotic syndrome, peritoneal infection, or malignancy [67]. Spontaneous bacterial peritonitis is an important complication of ascites and has to be differentiated from surgical cause of peritonitis such as perforation which accounts for 5% of cases [68]. Polymorphonuclear neutrophils (PMNs) of >250 mm^3 in ascitic fluid are suggestive of the presence of bacterial infection while predominant lymphocytic cells in ascites (>20% of the total leukocyte count) along with ascites–blood glucose quotient of <0.7 is suggestive of tuberculous infection. In the case of high PMNs in ascitic fluid with symptoms of fever and abdominal tenderness, antibiotics need to be continued even if the cultures are negative as 34.5% turn culture positive on repeated samples.

Management

Optimizing caloric and protein intake helps improving serum albumin and thus oncotic pressure. Diuretic therapy remains the first line of treatment in ascites. Aldosterone antagonist, spironolactone, is the initial drug of choice. It is usually started at 3 mg/kg/day in three to four divided doses and could be gradually increased to 6 mg/kg/day (maximum dose 400 mg/day). Potent loop diuretics such as furosemide (1–2 mg/kg) could be added if there is no effective diuresis on monotherapy. Spironolactone causes hyperkalemia while furosemide causes hyponatremia and hypokalemia, which has to be monitored on a regular basis. Sometimes duel therapy with these drugs is beneficial as potassium loss by furosemide is counteracted by potassium conservation by spironolactone. The suggested optimal drug dosage ratio to achieve this is 2 (furosemide):5 (spironolactone). Tolvaptan is an oral vasopressin receptor antagonist that helps in free water excretion and corrects hyponatremia, and it has been approved by the US Food and Drug Administration (FDA) for use in adults with cirrhosis. It is currently under phase three trials for its safety in children.

Paracentesis is indicated in the case of tense ascites causing respiratory distress. Usually, 20% albumin at a dose of 5 ml/kg is given slowly over 2 h during the procedure as plasma expander as well as to prevent rapid reaccumulation of ascites. It is shown that children tolerate large-volume paracentesis (>50 ml/kg) well, and the noticed side effect such as reduced urine output responded well to volume expanders [69]. It is advisable to taut the skin at the point of needle entry to create "Z," so that normal skin covers the muscle puncture site, preventing post-procedure leak.

Pulmonary Complications in Cirrhosis

The common pulmonary complications of CLD are HPS, portopulmonary hypertension (PoPH), and pulmonary hydrothorax. Care should be taken to exclude intrinsic lung diseases such as alpha-1-antitrypsin (AIAT) deficiency and cystic fibrosis that could coexist with liver disease and contribute toward hypoxia.

Hepatopulmonary Syndrome

Kennedy and Knudson coined the term "HPS" in 1977 to describe the association of cirrhosis with cyanosis and clubbing. The diagnostic criteria for HPS is the presence of CLD along with $PaO_2 < 70$ mmHg or alveolar-arterial oxygen gradient >15 mmHg and intrapulmonary vascular dilatation [70]. The overall prevalence of HPS ranges from 4% to 30% in cirrhotics. Suggested prevalence of HPS in pediatric population with CLD is around 8–20% [71, 72].

Pathogenesis

Higher prevalence of HPS in disorders where there is intrinsic liver disease along with PHT such as biliary atresia rather than in condition with PHT alone such as portal vein thrombosis led to the hypothesis that PHT along with intrinsic liver disease is essential for the development of HPS [52, 73, 74]. HPS is probably due to the effect of several vasoactive substances such as NO [17], carbon monoxide (CO), prostaglandins, vasoactive intestinal peptide, calcitonin, and glucagon, which escapes liver metabolism due to PHT and acts on pulmonary vasculature. NO is a potent vasodilator, and its nitrates and nitrite metabolites are found to be high in exhaled air of patients with HPS, which normalizes after liver transplantation. NO synthase is an enzyme that helps in production of NO from L-arginine. It has several isoforms of which eNOS was found to be increased in pulmonary small alveolar vessels, in small animal models of HPS. Elevated levels of ET-1 in blood and increased expression of its receptor ET B (ETB) expression in the pulmonary vasculature result in increased eNOS synthesis and thus vasodilatation [75]. Apart from eNOS, TNF-α, IL-1β, carbon monoxide, etc. are some of the other vasoactive substances implicated in pathogenesis of HPS [76–78]. These substances were thought to be involved in angiogenesis and vasodilatation of pulmonary vasculature leading to portopulmonary and hepatopulmonary shunts. In lung areas where there are capillary dilations, there would be more of perfusion compared to ventilation, while in areas with shunting effect, the blood is diverted away from alveoli resulting in less perfusion, leading to ventilation perfusion mismatch. Due to this, 100% oxygen inhalation might improve PaO_2 in cases of HPS with predominant intrapulmonary vascular dilatation, while there won't be any change in those with predominant shunt.

Clinical Manifestation

Dyspnea, clubbing, cyanosis, and spider nevi are some of the clinical manifestations of HPS. In HPS, dyspnea is more on upright position, due to increased congestion because of gravity exaggerating the ventilation—perfusion mismatch. PaO_2 decrease of 5% or more or 4 mmHg or more from the supine to upright position is defined as orthodeoxia, which is the hallmark of HPS [79]. Clubbing, also described as drumstick fingers (Fig. 74.4) seen in HPS, is due to the release of PDGF in nail beds that acts as growth factor and causes bulbous swelling of nail beds [80].

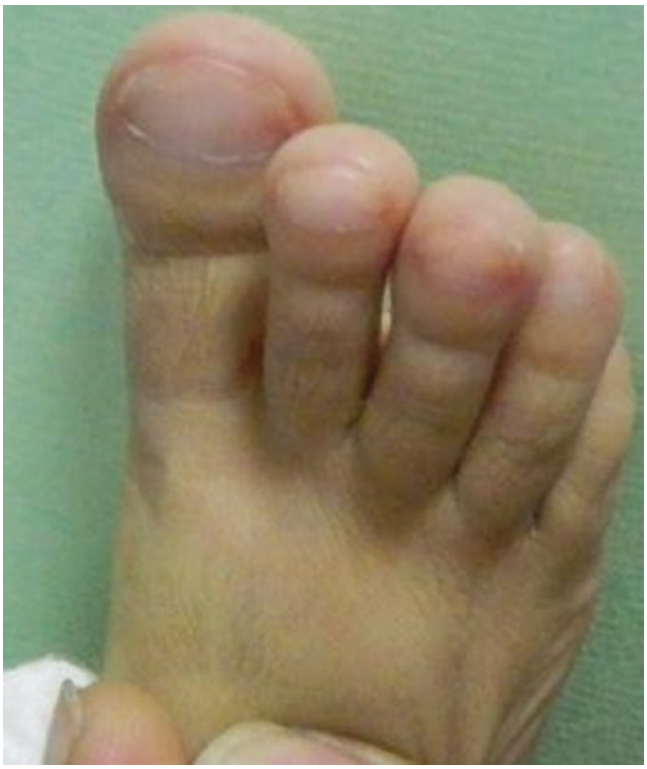
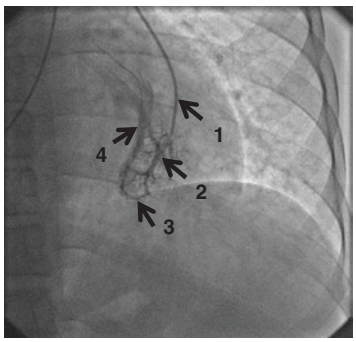


Fig. 74.4 Shows clubbing of fingers in a 10-month-old child with chronic liver disease due to missed biliary atresia



1. Catheter
2. Pulmonary artery
3. AV Shunt
4. Pulmonary vein

Fig. 74.5 Pulmonary angiogram showing AV Shunt

Diagnosis

In cirrhotic patients with hypoxia on arterial blood gas analysis, chest X-ray has to be taken as first line to rule out other causes of hypoxia such as pulmonary atelectasis, pneumonia, pulmonary edema, or hepatic hydrothorax. X-ray would be normal in majority of patients with HPS, but few might show interstitial infiltrate in the lung bases [81]. Based on ABG analysis in room air, severity of HPS could be graded as mild ($PaO_2 \geq 80$ mmHg), moderate ($PaO_2 = 60–79$ mmHg), severe ($PaO_2 = 50–59$ mmHg), and very severe ($PaO_2 < 50$ mmHg) [115].

Contrast-enhanced echocardiography is the preferred screening test for HPS [75]. Saline or indocyanine green is agitated to produce microbubbles at least 15 μm in diameter and then injected intravenously. These microbubbles act as contrast and could be visualized in echocardiography on the right side of the heart. When these microbubbles traverse the lung, they get trapped in alveolar microvasculature and gradually absorbed. In individuals with either intracardiac or intrapulmonary shunts, these microbubbles could be seen in the left heart. Differentiation between intracardiac or intrapulmonary shunts depends on the timing of appearance of microbubbles in the left heart. With intracardiac shunts, the microbubbles appear in three heartbeats, while with intrapulmonary shunts, it takes 4–6 heartbeats for the contrast to appear in the left side of the heart. Though contrast echocardiography is sensitive, it lacks specificity, as some cirrhotic patients with positive results on contrast echocardiography might not fulfill the diagnostic criteria for HPS [75].

If the initial screening raises the possibility of HPS, technetium-99 m macroaggregated albumin (Tc-99 m MAA) lung perfusion scan has to be considered, which is more sensitive and specific [70, 82]. Macroaggregated albumin, which is of 20 μm in size, is tagged with radioisotope technetium. In normal individuals, macroaggregated albumin gets trapped in the lung, and less than 5% of tracer activity can be quantified in the brain. In HPS patients, the fraction is more than 6%. Using this technique, the magnitude of shunt can be quantified which is inversely proportional to arterial oxygen saturation [70]. The drawback of this procedure is that correlation between shunt fraction and response of PaO_2 after 100% oxygen supplement remains unpredictable. High-resolution CT could show increased ratio of segmental arterial diameter to adjacent bronchial diameter, but published data were quite scarce [83]. Selective pulmonary angiography along with possible embolotherapy has to be considered in patients with HPS who fail to respond to 100% oxygen and particularly in those whom liver transplantation has to be considered. Angiography could reveal two types of vascular pattern, diffuse (type 1) and focal (type 2). In type 1 HPS, diffuse speckled, spidery, or sponge-like appearance of vasodilated vessels may be demonstrated. Type 1 was considered to be of better prognosis with liver transplantation as there is high possibility of resolution of HPS [84]. In type 2 HPS, vascular changes resembling arteriovenous (AV) shunts or vascular malformations could be seen. Figure 74.5 shows a discrete arteriovenous shunt in a child with HPS. If amicable, embolization of feeding vessel could be done before considering liver transplantation [85, 86].

Management

Oxygen supplementation remains the mainstay in HPS patients when $PaO_2 < 60$, and it improves the quality of life and exercise tolerance [87], but the usefulness of supplemental oxygen would wane away as HPS is a progressive disease. Several medications such as indomethacin, tamoxifen, somatostatin analogues, sympathomimetics, beta-blockers, methylene blue, and plasma exchange have been tried in HPS with abysmal results. Martinez-Palli et al. showed that decreasing PHT using TIPSS had no effect on pulmonary gas exchange and thus is not recommended [88]. Severity of HPS correlates with mortality, and so it should be combined with the model for end-stage liver disease (MELD) score as to prioritize these patients are in organ allocation [89, 90]. Liver transplantation is considered to be the definitive treatment in HPS patients as it reverts the pulmonary capillary abnormality and normalizes oxygenation [91]. Overall, postoperative complications in patients with HPS are higher than those without HPS. Embolic cerebral hemorrhage, worsening of hypoxia, and failure of AV shunts to resolve (particularly type 2) are a few of the unique complications of HPS [85, 92]. Careful patient selection is essential as severe preoperative hypoxemia ($PaO_2 < 50$ mmHg in room air) and significant intrapulmonary shunting (Tc-99 m MAA shunt fraction >20%) are associated with high mortality after liver transplantation [93]. Practical management protocol of hepatopulmonary syndrome during posttransplant (Tx) period is outlined in Fig. 74.6.

Portopulmonary Hypertension

PoPH is defined as the presence of mean pulmonary artery pressure (MPAP) > 25 mmHg along with pulmonary vascular resistance (PVR) > 240 dynes s cm^{-5} with normal capillary wedge pressure (PCWP) < 15 mmHg, in the presence of PHT [94]. It is postulated that the toxins and bioactive substances that bypasses liver metabolism via varices in PHT could lead to pulmonary vasoconstriction, structural remodeling of the

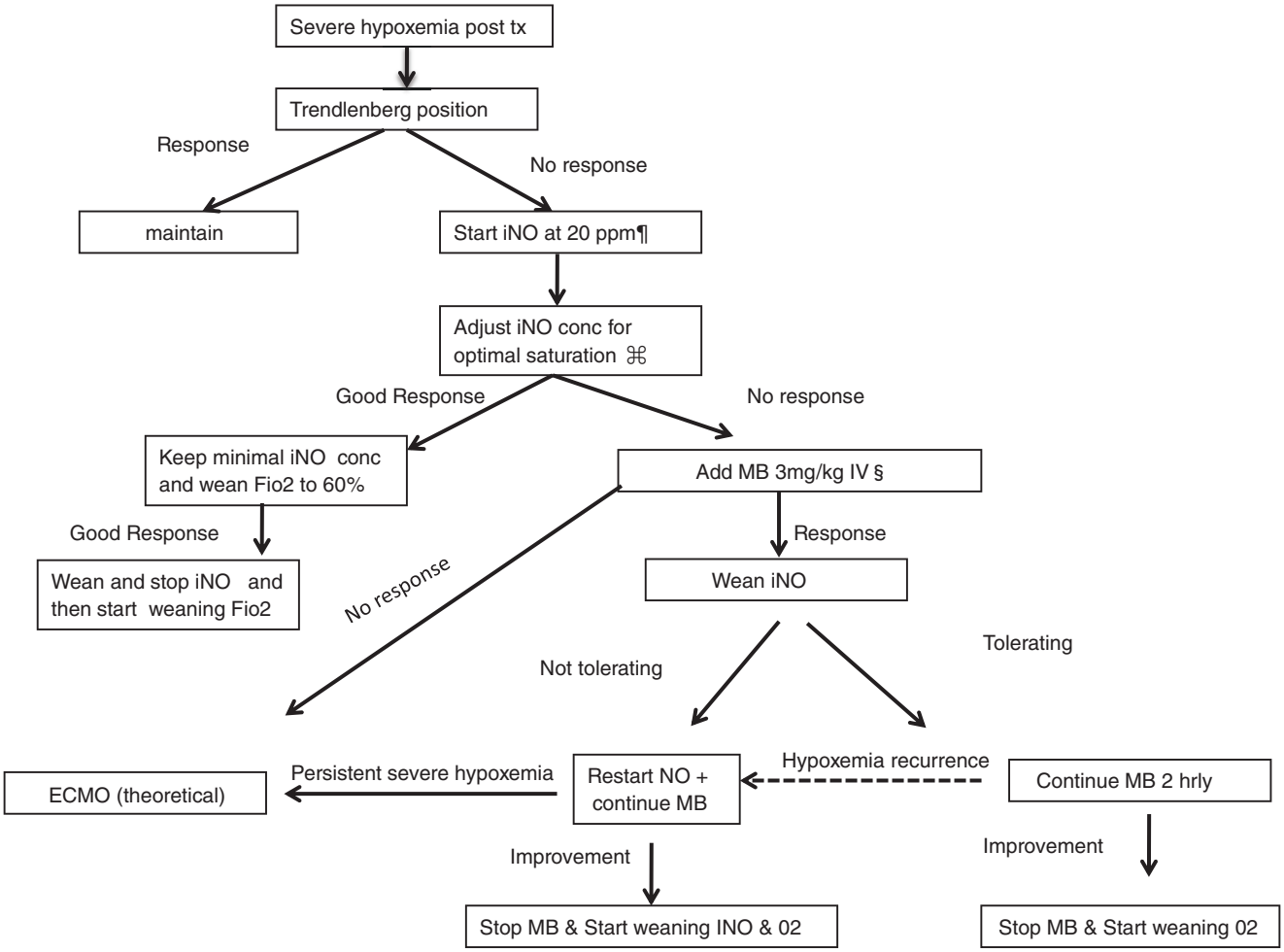


Fig. 74.6 Practical management protocol of hepatopulmonary syndrome during post transplant period. (Image barrowed with permission from Springer – License Number 4902070483558. Sundaram et al. [116])

pulmonary arteries, formation of microthrombi, etc. along with high flow state [95]. PHT seems to be a prerequisite as PoPH is seen in disorders with no intrinsic liver disease such as extrahepatic portal vein thrombosis [96]. The histopathological changes in PoPH consist of medial hypertrophy with nonspecific intimal fibrous thickening along with fibrous mural pads and occlusive fibrous tissue in vascular lumens, which is similar to that of primary pulmonary hypertension [53].

Clinical Features

The most common presenting symptom is dyspnea on excretion followed by syncope, chest pain, and fatigue [97]. Sometimes it is associated with hemoptysis. In mild-to-moderate PoPH, the symptoms are mild or absent. The time interval between diagnosis of PHT and symptoms due to PoPH is around 5 years [98]. Clinical examination would reveal systolic murmur with loud pulmonary component of second heart sound indicating tricuspid regurgitation. Edema and ascites (features of right heart failure) are other clinical features.

Diagnosis

Diagnosis of PoPH is made based on the presence of pulmonary hypertension in the background liver disease. Chest X-ray might show prominent central pulmonary arteries and cardiomegaly, and electrocardiogram might show right ventricular strain pattern. Estimated right ventricular systolic pressure >50 mmHg (normal <30 mmHg) is strongly suggestive of PoPH, when assessed by Doppler echocardiography, but has a false-positive rate of around 15% [94]. Right heart catheterization study remains the gold standard test for accurately diagnosing pulmonary hypertension and could be classified into mild, moderate, and severe based on MPAP, PVR, and cardiac index [94].

Management

Three groups of drugs, namely, prostacyclin analogues (epoprostenol, iloprost), ET receptor antagonists (bosentan), and phosphodiesterase inhibitors (sildenafil), have been used with good response in children with primary pulmonary hypertension, and the data on its usage in children PoPH are sparse [99]. Continuous intravenous infusion of epoprostenol (30–90 ng/kg/min) improves circulatory hemodynamics and exercise capacity in children with PoPH [99]. Treprostinil is another prostacyclin analogue, which could be given either subcutaneously or intravenously [100]. Bosentan is an ET receptor antagonist which when used in children has shown to decrease MPAP and pulmonary vascular resistance index and improve cardiac index [101]. Sildenafil is a phosphodiesterase-5 inhibitors and causes vasodilatation of pulmonary arteries and thereby decreases pulmonary hypertension when given at a dose of 0.5–1 mg/kg/dose given three to four times [99]. Mild-to-moderate PoPH frequently resolves after liver transplantation while severe PoPH is associated with the persistence of pulmonary hypertension and increased mortality [94].

Renal Involvement in Cirrhosis

Renal decompensation in liver disease is an important complication of both acute and CLD. The kidneys could be affected as a part of generalized circulatory changes associated liver disease (e.g., hypovolemia after variceal bleed) or as a specific phenomenon that occurs only in liver disease (e.g., hepatorenal syndrome). Renal involvement in liver disease has to be identified at the earliest and treated appropriately as progressive renal failure in liver disease is an adverse prognostic factor [102].

Hepatorenal Syndrome

HRS is a progressive, reversible functional renal impairment that occurs in patients with advanced liver cirrhosis or those with fulminant hepatic failure. Diagnostic criteria of HRS are outlined in Table 74.4. Type 1 HRS is characterized by rapid progression of renal failure and has a precipitating factor in most cases. It is associated with rapidly declining urine output and increasing creatinine in less than 2 weeks' duration. Type 1 HRS occurs in the setting of an acute deterioration of circulatory function (arterial hypotension and activation of the endogenous vasoconstrictor systems) and is frequently associated with rapid impairment in liver function and encephalopathy, carrying a poor prognosis. Type 2 HRS is characterized by a moderate renal failure, which follows a steady or slowly progressive course. It appears spontaneously in most cases and frequently associated with refractory ascites.

Pathophysiology

The hallmark of HRS is renal vasoconstriction, which progresses with worsening liver disease. The renal cortical blood flow has been documented to be reduced in HRS using selective renal arteriography and xenon-113 studies, but the underlying mechanisms involved in HRS are incompletely understood [103, 104]. Four pathways have been implicated in the pathophysiology of HRS.

Table 74.4 Six major criteria, all of which are necessary for the diagnosis of hepatorenal syndrome, and five additional criteria, which are usually associated with hepatorenal syndrome but are not required for diagnosis

Diagnostic criteria for hepatorenal syndrome[a]
The six major criteria are as follows
1. Presence of cirrhosis and ascites
2. Serum creatinine >1.5 mg/dL (133 μmol/L) or 24 h creatinine clearance of less than 40 mL/min
3. No improvement of serum creatinine (creatinine level £ 1.5 mg/dL or clearance >40 mL/min) after at least 48 h of diuretic withdrawal and volume expansion with albumin
4. Absence of shock, ongoing bacterial infection, fluid loss, or current or recent treatment with nephrotoxic medications
5. Absence of parenchymal kidney disease as indicated by proteinuria >500 mg/day
6. Absence of any evidence of obstructive uropathy on renal ultrasound scanning
The five additional criteria are as follows
Urine volume of less than 500 mL/d
Urine sodium concentration of less than 10 mEq/L
Urine osmolality greater than plasma osmolality
Urine RBC count of less than 50 per high-power field
Serum sodium concentration of less than 130 mEq/L

[a]As defined by the International Ascites Club

1. Peripheral and splanchnic vasodilation secondary to increased production of vasodilatory amines leading to activation of renin–angiotensin system in the kidneys, thereby reducing urine output.
2. Sympathetic nervous system activation leading to possible hepatorenal reflex.
3. Cardiac dysfunction contributing to renal hypoperfusion.
4. Action of cytokines and vasoactive mediators on renal circulation leading to the loss of renal autoregulation mechanisms may also play a role in the development of HRS.

Clinical presentation The incidence of HRS in patients with cirrhosis and ascites is 20% in the 1st year and 39% within 5 years. The rapidity and severity of renal failure at presentation depend on the type of HRS. Type 1 HRS is preceded by a precipitating factor in 70% of cases. The most common precipitating events are spontaneous bacterial peritonitis, large-volume paracentesis without volume replacement, gastrointestinal hemorrhage, and sepsis. These patients have many of the features present with advanced liver disease, in addition to renal dysfunction. These patients may also have underlying ascites, which are refractory to diuretic therapy. The findings in acute azotemia in patients with liver disease are given in Table 74.5.

Table 74.5 Features to differentiate prerenal azotemia, acute tubular necrosis, and hepatorenal syndrome

Differentiating hepatorenal syndrome from other causes of renal failure			
	Hepatorenal syndrome	Prerenal azotemia	Acute tubular necrosis
Urinary sodium concentration	<10 mEq/L	<10 mEq/L	<10 mEq/L
Urine plasma creatinine	>30:1	>30:1	<20:1
Urine osmolality	>Plasma osmolality	>Plasma osmolality	Equal to plasma osmolality
Fractional excretion of sodium	<1%	<1%	>2%
Urinary sediment	Normal	Normal	Casts, debris
Response to volume expansion	Brief or no diuresis	Sustained diuresis	No diuresis

Management

There is no proven effective therapy for HRS except liver transplantation. The general principles include treating precipitating factors and avoiding agents and factors, which can precipitate HRS. In cases of tense ascites, abdominal paracentesis done in conjunction with albumin replacement to avoid circulatory dysfunction has been proved to be safe [105]. Systemic vasoconstrictors including vasopressin analogues (terlipressin and ornipressin), somatostatin analogue (octreotide), and alpha-adrenergic agonists (midodrine and norepinephrine) are helpful in managing HRS. Studies have shown in adults that terlipressin when used alone or with albumin has higher efficacy in reversing the renal function in patients with HRS [106].

Portosystemic shunts such as TIPSS placement in adults have been shown to improve renal function. The mechanism by which TIPSS exerts this effect is still speculative but could be the result of reduction of portal pressure, suppression of a putative hepatorenal reflex, improvement of the circulating volume, or amelioration of cardiac function. Renal replacement therapy is indicated for those who failed to respond to vasoconstrictors, TIPSS, intractable metabolic acidosis, hyperkalemia, and volume overload and those who are waiting for a liver transplant.

Liver transplantation is the definitive treatment for patients with HRS, as it corrects liver dysfunction and eliminates PHT [30, 107]. Shusterman et al. showed that in 77% of those with HRS, serum creatinine levels decreased to less than 1.5 mg/dL after liver transplantation, and at 3 months post liver transplantation, none of the patients required dialysis [30, 107].

Other System Involvement in Cirrhosis

Pruritus is one of the most debilitating complications of CLD that affects the quality of life. Grading of pruritus and medical management is outlined in Table 74.6. Severe pruritus that does not respond to medical management per se is an indication for liver transplantation. Cardiac dysfunction in CLD is a discrete phenomenon called cirrhotic cardiomyopathy, and it is associated with all or some of following changes: (1) baseline increased cardiac output but blunted ventricular response to stimuli, (2) systolic and/or diastolic dysfunction, (3) the absence of overt left ventricular failure at rest, and (4) electrophysiological abnormalities including prolonged QT interval on electrocardiography and chronotropic incompetence [108]. Altered cardiac muscle membrane properties, impairment of stimulatory β-adrenergic receptor signaling pathways, and overactive negative inotropic factors are some of the factors implicated in pathogenesis of cirrhotic cardiomyopathy [109]. In a cohort of 40 children with median age of 8 months awaiting liver transplantation, Desai et al. showed that 27 (74%) had cirrhotic cardiomyopathy [110]. Liver transplantation remains the only curative therapy in most of the patients [110].

Increased caloric needs, decreased absorption due to cholestasis, and poor appetite contribute to undernutrition in CLD. Bone disease with both the components of osteoporosis and osteomalacia in CLD is termed as hepatic osteodystrophy (HO) [111]. The pathogenesis of HO is considered to be multifactorial due to complex interaction of IGF-1 deficiency, vitamin D and K deficiency, hypogonadism, etc., on growth and differentiation of bones in the background of hyperbilirubinemia [112]. The reported prevalence of fractures due to HO in children is around 10–13% [112]. Regular bone mineral density by dual-energy X-ray absorptiometry scan (DEXA) and monitoring serum 25-hydroxy vitamin D (25OHD) levels help in early detection and treatment of deficiency states. Routine supplementation of vitamin D at a dose of 3–10 times the recommended daily allowance has been suggested [60]. The recommended form of vitamin D for both prophylaxis and treatment is either ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) targeting a 25OHD level of more than 50 nmol/L. Bisphosphonate therapy is indicated only in the presence of low-impact fractures (≥1 vertebral, or ≥1 lower limb, or ≥2 upper limb) along with low bone mineral density (BMD) [113].

Table 74.6 Though grading is very subjective, it helps in comparing the symptomatic improvement to therapy

Grading and medical management of pruritus
Grade 1: no pruritus
Grade 2: mild scratching, but can be distracted
Grade 3: active scratching without abrasion
Grade 4: active scratching with abrasions
Grade 5: taneous mutilation with bleeding/scarring
Ursodeoxycholic acid (10 mg/kg twice a day)
Rifampicin (4–10 mg/kg/day)
Phenobarbitone (15–45 mg/day)
Naltrexone (0.1–0.5 mg/kg)
Ondansetron (0.1 mg/kg/three times a day to max 4 mg/dose)
Cholestyramine (1/3 to 1 sachet three times a day)

Prognosis

MELD is a scoring system developed to predict death within 3 months of surgery in patients who had undergone a TIPSS procedure and was subsequently found to be useful in determining prognosis in patients with CLD due to other etiologies. MELD is calculated using serum bilirubin, serum creatinine, and international normalized ratio (INR). A value of 40 or more is associated with 71% mortality in 3 months, while a value of less than 9 is associated mere 1.9% mortality. MELD is used in the USA and European countries in prioritizing CLD patients for liver transplantation. In children under 12 years of age, pediatric end-stage liver disease (PELD) score is used for prognostication where age less than 1 year and growth failure are also taken into consideration. Though many countries use PELD to prioritize children in waiting list, it has its own limitations. PELD score does not take into consideration complications such as cholangitis, severe PHT, pulmonary hypertension, and HPS and could underestimate the risk of death [17].

Conclusions

Cirrhosis secondarily affects nearly every system in the body, where the correlation might or might not be proportional to the severity of liver disease. Apart from managing complications of cirrhosis, it is essential to take care of the general well-being of these children such as growth monitoring, immunization, vitamin supplementation, and their education. Though the functionality of end organs (brain, kidney, lungs, etc.) could revert back to normal or near normal after liver transplantation, advanced end organ damage might render a patient unfit to undergo liver transplantation. Anticipation of the complication and timely intervention would help in better outcome in these children.

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Nutritional Management of Children with Liver Disease

75

Sara Mancell

Introduction

The liver is the key organ in the production and distribution of nutrients, playing a central role in glucose homeostasis, protein synthesis, bile salt production, lipid metabolism and vitamin storage. Chronic liver disease can disrupt these processes leading to malnutrition. Malnutrition is highly prevalent in children with liver disease and is associated with increased morbidity and mortality [1]. Potential causes of malnutrition include insufficient nutrient intake, increased nutritional requirements, impaired nutrient absorption and altered metabolism. Nutrition strategies are centred on managing these factors while promoting normal growth and development. Maintaining optimal nutrition in children with chronic liver disease may prevent further liver damage and improve outcomes post-transplant [2].

In acute liver disease, if the presentation is rapid, children may be well nourished initially. The aim is to preserve nutritional status and manage complications such as hypoglycaemia and hyperammonaemia.

The nutrition management of liver disease caused by inborn errors of metabolism does not form part of this chapter.

Dietary Assessment

The nutritional assessment and monitoring of patients with liver disease is essential, in order to assess requirements as well as to evaluate progress. A dietetic assessment should include the current situation and relevant history. It should be carried out at regular intervals to take account of changes in clinical status, nutrient intake, social factors, activity levels and anthropometry (Table 75.1).

Table 75.1 Anthropometric measurements

Height	Good indicator of long-term nutritional status <2 years = measure using a length board >2 years = measure using a stadiometer
Weight	Good indicator of short-term nutritional status Affected by organomegaly/ascites. Weigh infants naked and older children with no shoes and light clothing
Abdominal girth	Useful adjunct where there is organomegaly/ascites, especially where weights are fluctuating
Head circumference	Good indicator of long-term nutritional status Only used in children under 2 years
Mid upper arm circumference (MUAC)	Good indicator of short-term nutritional status Measure the same arm each time High inter-observer error so use a single observer ideally
Triceps skinfold thickness (TSF)	Good marker of medium- to long-term nutritional status Practically difficult unless the child is cooperative High inter-observer error so use a single observer ideally

Nutritional Management of Chronic Liver Disease

The causes of malnutrition may be multifactorial (Fig. 75.1). Nutrition strategies are focused on each of these areas.

Decreased Nutrient Intake

Poor intake of nutrients may be due to the following:

- Nausea and vomiting
- Pruritis
- Taste changes associated with medications

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- Unpalatable diets/specialist feeds
- Reduced gastric capacity and discomfort from ascites or organomegaly
- Behavioural feeding difficulties
- Fluid and sodium restriction due to ascites
- Investigations and procedures interrupting meals or feeds
- Anorexia

Feeding strategies should take into account the causes of poor nutrient intake which may be multifactorial. When oral intake is significantly reduced because of persistent nausea or vomiting or the discomfort of pruritis or unpalatable feeds, supplemental tube feeding may be required. This can be given during the day as bolus feeds and/or as a continuous overnight infusion. Where there is ascites or organomegaly, high energy, low volume feeds may help with the problem of reduced gastric capacity. With ascites, a rigid sodium restriction is not recommended, but rather a general reduction is salty foods. In hospitals, extended periods of nil by mouth and feeding interruptions due to investigations and procedures should be kept to a minimum, and every effort should be made to make mealtimes enjoyable.

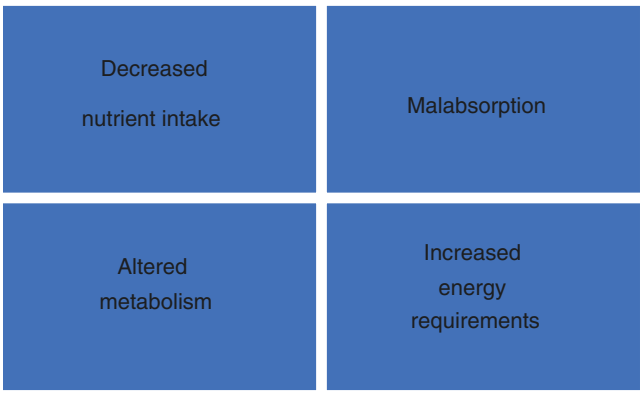

Fig. 75.1 Causes of malnutrition in liver disease

Malabsorption

Malabsorption in chronic liver disease may result in poor growth and fat-soluble vitamin deficiency and may be due to the following:

- Absent or reduced bile flow
- Pancreatic insufficiency
- Portal hypertension causing mucosal oedema

Long-chain triglycerides require emulsification by bile in the intestine, and therefore the absence or reduction of bile flow may lead to fat malabsorption. An energy-dense diet is given combined with medium-chain triglyceride (MCT) supplementation. MCTs do not require emulsification with bile, diffusing easily into the intestinal cells where they are absorbed directly into the bloodstream. There is little evidence regarding how much dietary fat should be MCT with recommendations varying between 30% and 75% [1, 2]. Table 75.2 shows commonly used formulas, feeds and supplements containing MCT.

In some types of liver disease such as Alagille's syndrome, malabsorption may result from pancreatic enzyme

Table 75.2 Formulas, feeds and supplements containing medium-chain triglycerides

Feed per 100 ml	Age (years)/ weight	Dilution (%)	Energy (Kcal)	Protein (g)	Carbohydrate (g)	Fat (g)	Protein type	MCT (%)	Comments
Heparon Junior®	0–3 y	18	86	2	11.6	3.6	Whole	49	Supplemented with BCAA
Aptamil Pepti Junior®	0–1 y	13.4	66	1.8	7.2	3.4	Peptide	50	
Pregestimil Lipil®	0–1 y	13.5	68	1.89	6.9	3.8	Peptide	55	Clinically lactose-free (<5 mg lactose/100 mL)
Puramino	0–1 y	13.6	68	1.89	7.2	3.6	Amino acid	33	Clinically lactose-free (<5 mg lactose/100 mL)
Infatrini Peptisorb®	0–1.5 y or <9 kg	n/a	100	2.6	10.3	5.4	Peptide	50	
Peptamen Junior®	1–10 y	22	100	3	14	3.8	Peptide	53	
Paediasure Peptide®	8–30 kg	n/a	100	3	13	4	Peptide	50	
Nutrini Peptisorb	1–6 y or 8–20 kg	n/a	100	2.8	13.6	3.9	Peptide	46	
Nutrison MCT	>6 y	n/a	100	5	12.6	3.3	Whole	61	
MCT Oil	From birth	n/a	855	0	0	95	n/a	100	Not a complete source of nutrition
Liquigen	From birth	n/a	450	0	0	50	n/a	96	Not a complete source of nutrition
MCT Procal (per 100 g)	>3 y	n/a	703	12.2	0	63.5	Whole	96	Not a complete source of nutrition

insufficiency [3] and supplementation with pancreatic enzymes may be required. Inflammation and mucosal oedema in the small bowel due to portal hypertension may cause protein malabsorption [4] necessitating a change to a feed with hydrolysed protein.

Altered Metabolism

As liver failure progresses, the metabolism of carbohydrate, protein and fat may be altered. There is a reduction in glycogen storage and an increased breakdown of fat and protein to meet energy demands alongside inefficient use of the substrates which are available [5]. Mager et al. [6] showed that there is increased oxidation and therefore an increased requirement for branched chain amino acids (BCAA) even in children with relatively mild liver disease. The result of these changes is wasting of fat and lean body mass, hypoglycaemia, hypoproteinaemia and hyperammonaemia [7], and dietary strategies are centred on reversing or preventing these.

Preserving Lean Body Mass

To minimise catabolism, periods of fasting should be limited so that an exogenous supply of nutrients is used in preference to body fat and muscle stores. This can be achieved by giving feeds or meals more frequently or using a feeding pump for continuous feeding. Chin et al. [8] found that supplementation with BCAAs (valine, leucine, isoleucine) may help to improve nutritional status in liver disease, although the evidence for this is limited.

Hypoglycaemia

Where there is hypoglycaemia, the amount of carbohydrates or the times that they are given may need to be adjusted. The carbohydrate content of formulas can be increased by adjusting the concentration of powdered formulas, using a high energy feed or adding glucose polymers (Table 75.4). In older children, the inclusion of starchy carbohydrates and bedtime snacks may be necessary. Intravenous dextrose and close monitoring of blood sugars may be required, especially during periods of fasting or illness.

Hyperammonaemia

A major site of ammonia production is the large intestine, where protein is broken down by bacteria to produce ammonia. Ammonia is converted to urea in the liver, which is then excreted by the kidneys. In liver failure, this process is disrupted resulting in accumulation of ammonia in the blood. Exposure of brain tissue to toxic levels of ammonia leads to hepatic encephalopathy. A dietary protein restriction may be required for the first 24–48 hours [9], but a prolonged protein restriction may worsen catabolism, resulting in reduced muscle mass. This may further worsen hyperammonaemia as muscle is an important site of ammonia uptake.

Increased Energy Requirements

Children with end-stage liver disease may have increased requirements due to the following:

- Stress factors such as infection
- Increased respiratory effort from ascites or organomegaly
- Increased metabolically active cells mass

Greer et al. [10] found that resting energy expenditure (REE) was almost 30% higher than controls and suggested that this was because children with end-stage liver disease have less body fat and a higher relative proportion of metabolically active cells. In contrast, Kyrana et al. [11] found that there was no difference in REE between children with end-stage liver disease and healthy controls and recommended individualised assessments of REE rather than assuming hypermetabolism. Unfortunately, measurements of REE are often impractical in the clinical setting. Given the possibility of an increased REE combined with malabsorption, estimated energy and protein requirements for children with chronic liver disease are high (Table 75.3). Requirements are calculated based on the individual, taking into account age, sex, nutritional status, disease state and growth. Calculated requirements are used only as a guide and are not static; they should be re-calculated as part of the dietary assessment.

There are several methods for increasing the nutrient density of the diet to achieve high energy and protein requirements, including concentrating powder-based feeds, using nutrient-dense feeds and supplements and encouraging high energy/protein meals and snacks (Table 75.4).

Table 75.3 Energy and protein requirements used in practice for children with chronic liver disease

	Infants	Older children
Energy	120–150 kcal/kg	130–150% of the estimated average requirement for age
Protein	3–4 g/kg	130–150% of the recommended nutrient intake

Table 75.4 Strategies to increase energy and protein intake

Concentrating feeds	The concentration of a powder-based formula is increased beyond the recommended dilution, usually in 2% increments e.g. Heparon Junior® 18% = 86 kcal, 22% = 106 kcal/100 ml This also increases micro/macro-nutrients, renal solute load, osmolality, risk of osmotic diarrhoea Explicit instructions needed to avoid preparation errors (usually provided by a registered dietitian)
Nutrient-dense feeds and supplements	Nutrient-dense feeds/supplements orally or via tube Where non-protein energy sources (glucose polymers, fats) are added to the diet, care should be taken to maintain an acceptable protein-energy ratio
Nutrient-dense meals and snacks	High energy/high protein foods e.g. cream, avocado, nuts, nut butters, cheese, butter, oil

Table 75.5 Fat-soluble vitamin supplementation

Vitamins	
Multivitamin (including A and D)	<1 year: Abidec or Dalivit 0.6 ml >1 year: Abidec or Dalivit 1.2 ml >12 years: Forceval 1 capsule
D	1–12 months: Colecalciferol 1000 IU (maximum 3000 IU/day) 1–12 years: Colecalciferol 6000 IU in solution or 6400 IU if able to swallow capsules (maximum 25,000 IU/day) >12 years: Colecalciferol 9600 IU daily (maximum 40,000 IU daily)
E	0–12 years: Alpha tocopheryl 10 mg/kg up to 100 mg/kg (maximum 200 mg/kg per day) >12 years: Alpha tocopheryl 286 mg (maximum 200 mg/kg/day)
K	<1 year: Phytomenadione 1 mg 1–5 years: Phytomenadione 2 mg 5–12 years: Menadiol tablets 5 mg >12 years: Menadiol tablets 10 mg

Fat-Soluble Vitamin Supplementation

Table 75.5 shows how fat-soluble vitamins are supplemented for cholestasis at the author's institution. It should be noted that these are suggested starting doses, and prescribed amounts should be adjusted according to serum levels of fat-soluble vitamins. If serum vitamin levels are particularly low, they may be given intra-muscularly.

Methods of Feeding

Oral Feeding

All children should be encouraged to feed orally where possible. Behavioural feeding difficulties are common in children who have gastrointestinal-related aversive experiences such as abdominal discomfort and vomiting [12] or extended periods with no oral feeding [13], all of which may occur with chronic liver disease. With infants, it is especially important to encourage age-appropriate weaning practices, to avoid missing the 'window of opportunity' to introduce tastes and textures [14]. This can be especially difficult when there is ascites or organomegaly or when the child is being tube fed. The emotional and social benefits of eating and drinking for the child and family are also important, and eating should be encouraged, even if oral intake is minimal.

Tube Feeding

Tube feeding may be required in children who are unable to take sufficient nutrition orally. Nasogastric tube feeding has been associated with improved body composition [15] and growth [16] in children with liver disease. Tube feeding may have a positive impact on quality of life as it can remove the pressure on both the child and family to meet requirements orally. The tube can be used to administer unpalatable feeds and medicines, and continuous or frequent feeds can minimise periods of fasting, thus helping to maintain blood sugars and preserve body stores.

Gastrostomy feeding is preferred in children with long-term feeding problems but is rarely possible in children with chronic liver disease. Portal hypertension and intra-abdominal varices increase the risk of bleeding during placement of the tube, while ascites may prevent adequate tract formation around the gastrostomy [17]. Gastrostomy feeding has been used successfully in children with no portal hypertension, varices or ascites [18].

Parenteral Nutrition

Due to the risks associated with parenteral nutrition (PN), PN should only be used when it is not possible or effective to feed enterally [19]. For example, PN may be used when NG placement is not possible (e.g. due to large, bleeding varices) or where there is significant and persistent malabsorption impacting on growth. Wendel et al. [20] found that there was an improvement in nutritional status in children with end-stage liver disease awaiting transplant who were on PN as they were unable to tolerate enteral nutrition.

The Management of Common Liver Conditions

Conjugated Hyperbilirubinaemia

Infants presenting with conjugated hyperbilirubinaemia will generally require MCT supplementation. If galactosaemia

has not been excluded, breast feeding should be stopped and replaced with an MCT feed containing only trace amounts of galactose (e.g. Pregestimil®). The mother should be encouraged to express breast milk as it is usually possible to restart breast feeding once galactosaemia is excluded. If cholestasis persists with accompanying symptoms of malabsorption and suboptimal growth, MCT supplementation will continue to be required. This can be given as 2–5 ml of a fat emulsion with every breast feed or as an MCT-containing formula (Table 75.2) either exclusively or alongside some breast feeding.

Biliary Atresia

Biliary atresia presents in infancy with jaundice due to biliary duct obstruction and is managed surgically with a Kasai portoenterostomy to restore bile flow [21]. The nutritional intervention is as described for conjugated hyperbilirubinaemia. Close monitoring is important as BA progresses to end-stage liver disease at some point in the majority, with 50% of infants requiring a liver transplant before age two and 75% by age 20 [21]. Standard weaning practices are advised, with age-appropriate solids. MCT supplementation continues until jaundice has cleared or if jaundice does not resolve, MCT supplementation may continue until transplant.

Non-alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) exists as a spectrum ranging from benign hepatic steatosis to more aggressive forms that can potentially progress to cirrhosis in childhood [22]. It is the most common liver abnormality in the paediatric population [23]. Weight reduction through dietary modification and physical activity is the cornerstone of management. The aims are to reduce insulin resistance and visceral obesity to decrease oxidative stress [24]. A study of 84 children with NAFLD, who underwent a 12-month diet and lifestyle programme, demonstrated a significant reduction in BMI, fasting glucose levels, insulin, lipids and liver enzyme activity [22]. Evidence for other treatments including vitamin E, metformin and ursodeoxycholic acid have produced equivocal results. Lifestyle changes remain the mainstay of treatment.

Wilson's Disease

Wilson's disease is an autosomal disorder of copper metabolism and may present at almost any age. Copper accumulates in the liver during childhood but may also deposit in other parts of the body such as the brain, eyes, joints and kidneys. Treatment is primarily through chelating agents that bind dietary copper for excretion. Foods with very high concentrations of copper such as offal, shellfish, nuts, dried fruit, chocolate and mushrooms may need to be avoided [25] although this is usually only necessary in patients who are unresponsive or non-compliant with drug therapy [26].

Progressive Familial Intrahepatic Cholestasis

Progressive familial intrahepatic cholestasis (PFIC) refers to a group of autosomal recessive disorders that interfere with the secretion of bile and often present in infancy with cholestasis [27]. The major problem from a nutritional point of view is fat malabsorption, necessitating MCT supplementation. Fat malabsorption may occur without jaundice due to the inability of patients with PFIC to produce bile salts. Poor intake and appetite may also occur as a result of intractable pruritis. Short stature is common, though may improve following liver transplantation [28]. Supplementary nutrition support via nasogastric tube or gastrostomy is often required.

Alagille's Syndrome

Alagille's syndrome can be particularly challenging from a nutrition point of view. It is characterised by cholestasis and malabsorption, poor growth, selective eating, intractable pruritis, renal acidosis [29] and pancreatic insufficiency in some patients [30]. The nutritional management is as described for conjugated hyperbilirubinaemia. Intervention through specialist feeding clinics may be beneficial to address selective eating. Supplementary nutrition support via nasogastric tube or gastrostomy is often required.

Intestinal Failure-Associated Liver Disease

Intestinal failure-associated liver disease (IFALD) is seen in 40–60% of children with intestinal failure on parenteral nutrition [31]. It can progress from cholestasis to fibrosis and cirrhosis and result in the need for liver and/or small bowel transplantation. It may result because of physiological abnormalities associated with intestinal failure or from the toxic effects of PN [32]. Risk factors include prematurity, short bowel syndrome, sepsis, intestinal bacterial overgrowth and a lack of enteral nutrition [33]. Strategies to reduce the risk of IFALD include early implementation of enteral feeding, a specialised, multidisciplinary approach and techniques focused on avoiding sepsis [34]. The use of the lipid formulation SMOF (soybean oil, MCT, olive oil, fish oil) in place of soybean oil has been a significant advancement and has been shown to reduce IFALD-related cholestasis [35].

Liver Transplantation

Feeding post-transplant usually starts within 3–5 days, via nasogastric tube and then progresses fairly quickly to a normal oral diet. High energy feeds are used for patients with poor nutritional status with catch-up growth typically seen in the 2 years following transplant. However, many children may achieve a final height below their genetic potential [36]. Where there are gastrointestinal complications (e.g. bowel perforation) or severe undernutrition at the time of transplant, parenteral nutrition may be required. Pre-existing behavioural feeding difficulties are common and may mean that tube feeding continues for an extended period. In cases where children are likely to continue to require long-term tube feeding post-transplant, the aim will be to keep the current gastrostomy or place a new one during transplant where possible.

Following liver transplant, Seville oranges and grapefruit should be avoided as they may interfere with immunosuppressant medication. It is important to adhere to food safety guidelines and avoid foods that may contain bacteria such as listeria, E. coli or salmonella as there is an increased vulnerability to food poisoning when on high dose immunosuppressant medication.

Chylous Ascites

Chylous ascites is a potential complication post liver transplant. It usually occurs as a result of damage to the lymph vessels during surgery and results in a loss of chyle, a milky, triglyceride-rich fluid, into the peritoneal cavity. This is often evident in the intra-abdominal drain. Treatment for chylous ascites involves the dietary restriction of long-chain triglycerides (LCT) to reduce the flow of lymph in the disrupted lymphatic system. Dietary restriction generally lasts for at least 3 weeks. A feed or formula with high levels of MCT may be used (Table 75.6) alongside low LCT foods. As MCTs are not a source of essential fatty acids (EFAs), supplementation with EFAs may be required (e.g. with walnut oil).

Transplant-Acquired Food Allergy

Transplant-acquired food allergy (TAFA) is present in as many as 17% of transplanted children [37]. The cause of TAFA is not known but may be due to increased permeability of the intestine due to immunosuppressants or the transfer of lymphocytes or IgE as a result of the transplant [38]. Some children may need a long-term dietary restriction, whereas others do appear to develop tolerance over time [39].

The Nutritional Management of Acute Liver Failure

The nutritional consequences of acute liver failure (ALF) include catabolism, hypoglycaemia, hyperammonaemia, hepatic encephalopathy, electrolyte, acid base and fluid disturbance and conjugated hyperbilirubinaemia. Catabolism results from impaired glycogen storage and gluconeogenesis with protein and fat stores broken down to meet energy demands [9]. The catabolic effect is increased by a rise in insulin and glucagon, driving the need for gluconeogenesis to maintain blood sugars [40] and an increase in resting energy expenditure [41]. Hypoglycaemia can worsen the neurological prognosis [42] and is associated with poor outcomes and mortality [43]. Hyperammonaemia occurs due to impaired detoxification of ammonia to urea with increased aromatic and decreased BCAAs in circulation [9]. Management is based on preventing hypoglycaemia, managing metabolic disturbances such as hyperammonaemia, maintaining nutritional status despite fluid restrictions imposed in the critical care setting and supplementing the diet with MCT where there is jaundice (Table 75.2).

Metabolic disorders are a frequent cause of ALF and require specific dietary therapy to prevent accumulation of toxic by-products. The ideal dietary therapy cannot begin until the diagnosis is determined, which can take time.

Table 75.6 MCT feeds which are suitable for chylous ascites

Feed per 100 ml	Age	Standard dilution (%)	Energy (Kcal)	Protein (g)	Carbohydrate (g)	Fat (g)	Protein type	MCT (%)	Comments
Monogen	From birth	17.5	74	2.2	11.6	2.2	Whole	82	
Low Fat Module	From birth	18	67	1.6	14.9	0.14	Whole	100	Contains no essential fatty acids.
Lipistart	0–10 years	15	70	1.8	8.7	3.2	Whole	74	
Emsogen	>1 year	20	88	2.5	12	3.3	Amino acid	83	For cow's milk allergy or when amino acid required

Summary

Optimal nutrition support which is individually tailored and responsive to the changing clinical picture is essential for children with liver disease. Nutrition therapy should focus not only on correcting nutritional deficits and managing the complications of liver disease but also promoting normal growth, development and quality of life.

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Paediatric Liver Transplantation

76

Annalisa Dolcet and Nigel Heaton

Introduction

Liver transplantation continues to be the only effective treatment for children with end-stage liver disease. Thomas Starzl performed the first liver transplant in a child in March 1963 [1]; however, it was not until 1967 that he reported the first recipient with significant survival [2]. Following his early series of 7 children aged between 13 months and 16 years [2], more than 15,000 paediatric liver transplants have been carried out in the USA and 10,000 in Europe, with 3- and 5-year survival of 80% and 75%, respectively.

There have been continued improvements in all aspects of care of the child with liver disease coming to liver transplantation including surgical, anaesthetic, intensive care and postoperative management and long-term graft, and patient survivals are now exceptionally good with improved quality of life (QOL) for recipients. The advent of reduced size and split LT in the 1980s led to a significant decrease in waiting list mortality for children. The paediatric overall waiting list mortality is about 10% and appears to be highest in children younger than 6 years. The change in donor demographics over the past 25 years has resulted in a reduction in the number of brain-dead donor livers suitable for splitting, which is the commonest graft for young children in the West. Living donor liver transplantation (LDLT) was first performed in 1989; and subsequently developed as perhaps the most common form of organ donation in the East [3, 4]. Most recently, liver perfusion machine has been utilized to try to improve the condition of marginal donor livers. To date this has not impacted on paediatric liver transplantation, but with increasing experience, this is likely to change.

The large numbers of recipients currently surviving beyond 20 years are informing clinical practice and providing more information for families currently facing transplantation. As the majority of children are transplanted at a young age and have little memory of the events surrounding their transplant, continuing patient and family education is increasingly recognized as important. There has been a change of emphasis in care from a focus on survival to long-term outcomes centred on well-being, psychosocial and physical development and educational attainment. It is clear that adolescence and transition to adulthood and follow-up within an adult environment pose further significant challenges and late death due to non-adherence to medication and follow-up are significant problems. The emergence of models of care to manage these challenges will hopefully help lead to further improvement in long-term outcomes. In addition, the timing of transplant and its influence on subsequent development and outcome is coming under increasing scrutiny.

Pre-transplant

Historically children have been listed for liver transplantation based on criteria adopted from adult experience. However, children present with a different spectrum of diseases, with two-thirds of children coming to liver transplantation in the first 5 years of life, and consideration has to be given to their emotional, social, intellectual and physical development. The timing of liver transplantation has to be considered with the long-term development of the child in mind, and there remains a lack of data regarding this topic.

Indications for Liver Transplantation

Liver transplantation should be considered for any child with end-stage liver disease with a predicted prognosis of less

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than 18 months, acute liver failure (ALF), unresectable hepatic tumours and liver-based metabolic defects.

Indications for liver transplantation are in general derived from adult liver transplant experience but are modified for children and include:

- Liver decompensation (prolonged INR, low serum albumin, ascites)
- Disordered metabolism (jaundice, loss of muscle mass, osteoporosis)
- Portal hypertension (variceal bleeding, severe hypersplenism, intractable ascites)
- Encephalopathy
- Spontaneous bacterial peritonitis
- Hepatopulmonary syndrome, hepatorenal syndrome
- Pulmonary hypertension
- Recurrent cholangitis and intractable pruritus
- Quality of life (failure to growth, poor concentration, lethargy)
- Tumours

Extrahepatic biliary atresia is the most common indication for liver transplantation and accounts for 40–50% of cases listed worldwide. Common indications for liver transplantation are listed in Box 76.1. The majority of paediatric recipients under 2 years old have cholestatic diseases, particularly biliary atresia, which accounts for 74% of cases in this age group. Metabolic disorders and ALF are less common indications and account for 9% each of the overall number [5]. However, metabolic diseases, although individually rare, collectively are becoming a more common indication for transplantation.

Box 76.1 Indications for liver transplantation in children

- *Traditional*
  - Cholestatic conditions
    - Biliary atresia
    - Sclerosing cholangitis
    - Parenteral nutrition-associated cholestasis
    - Alagille syndrome
    - Progressive familial intrahepatic cholestasis (BSEP and MDR3 deficiency)
  - Metabolic disease
    - Alpha 1 antitrypsin deficiency
    - Cystic fibrosis
    - Gestational alloimmune liver disease
    - Urea cycle defects
    - Tyrosinemia
    - Wilson disease
    - Primary hyperoxaluria
    - Glycogen storages disorders
  - Tumours
    - Hepatoblastoma
    - Haemangioendothelioma
    - Hepatocellular carcinoma
  - Acute liver failure
  - Cryptogenic cirrhosis Budd-Chiari syndrome
  - Propionic acidaemia
- *Controversial*
  - Mitochondrial hepatopathy
  - Metastatic liver tumours
  - Progressive familial intrahepatic cholestasis (PFIC1 deficiency)

Box 76.2 Contraindications to liver transplantation

- Absolute
  - Extrahepatic malignancy
  - Irreversible "severe" neurological injury
  - Multisystem organ failure
  - Active uncontrolled sepsis
  - Uncorrectable life-limiting defects in *other* critical organs – Kidney – Heart – Lungs

Chronic Liver Diseases

Biliary Atresia

Extrahepatic biliary atresia (BA) is a destructive inflammatory obliterative cholangiopathy that affects the intrahepatic and extrahepatic bile tree. Type 3 BA is the most frequent form of the disease accounting for 90% of cases and is the most severe form with a solid porta hepatis, microscopic ductules and a solid gallbladder or mucocele [6]. The majority of children coming to transplant have undergone Kasai porto-enterostomy within the first 3 months of life. Early porto-enterostomy and expertise of the multidisciplinary team have a significant impact on outcome and the need for liver transplantation in early life [7, 8]. The results of concentrating expertise in a small number of centres each performing more than 5 cases per year in the UK have led to a 4-year survival with the native liver intact of 41–51% and an overall survival of 87–89%. More recently survival of 96% at 10 years has been reported for the UK with an integrated programme of Kasai porto-enterostomy and liver transplantation [6]. Mortality is distributed equally between deaths on waiting list for liver transplant and in the post-transplant period. By the age of 18 years, approximately 80% of children with BA will have been treated by liver transplantation. Outcomes have been reported for 5- and 10-year actuarial graft and patient sur-

vival of 76.2% and 72.7% and 87.2% and 85.5% for cadaveric [7] and 84.9% and 76.6% and 86.7% and 80.8% for LDLT [9], respectively.

The majority of young children (under 5 years of age) with BA will come to transplant with jaundice and synthetic failure. In a small number of children (6% of cases), acute decompensation secondary to ischaemic hepatitis may occur following a viral illness or infection. Children at risk of ischaemic hepatitis and liver decompensation are those with a hepatic artery resistance index of greater than 1 on Doppler ultrasound where the liver is dependent on arterial inflow [10]. An episode of systemic hypotension will lead to arterial insufficiency, and the liver will take an ischaemic "hit" and precipitate acute liver decompensation. Urgent liver transplantation will rescue these children if recognized in time. Children older than 5 years of age may present with failure to grow and a falling serum albumin (synthetic failure), but without jaundice. Adolescents coming to transplantation will invariably have portal hypertension as a dominating feature, which in association with adhesions from previous surgery can make for a difficult surgical challenge. The presence of portosystemic collaterals, particularly in the presence of a small or thrombosed portal vein, requires surgical ligation at the time of surgery to maintain PV flow postoperatively.

Congenital anomalies associated with "syndromic" BA (15% of all cases) include polysplenia/asplenia, absent inferior vena cava, portal hypoplasia, preduodenal portal vein, malrotation and situs inversus and may complicate surgery and influence graft choice.

Cholestatic and Metabolic Disorders

Cholestatic liver diseases excluding BA account for 10% of liver transplants in children. These include Alagille syndrome, progressive familial intrahepatic cholestasis and sclerosing cholangitis. Liver transplantation is often used to treat symptoms, such as severe pruritus. Children with Alagille syndrome are at risk of growth failure and morbidity from pruritus, xanthomas and complications of vitamin deficiency. Progressive familial intrahepatic cholestasis (PFIC) defines a group of disorders characterized by chronic, unremitting cholestasis and autosomal recessive inheritance with a shared pattern of biochemical, clinical and histological features. Liver transplantation is reserved for those with severe symptoms including pruritis or progressive liver disease. Earlier transplant may lessen future growth and developmental impairment in some, but not all of these conditions [11]. In Alagille syndrome, the biliary hypoplasia is associated with other congenital malformations, the most important of which is pulmonary artery stenosis. This needs to be assessed preoperatively due to the risk of mortality post reperfusion if cardiac output is limited by the pulmonary stenosis. Dobutamine stress testing has been used to identify at risk children who are unable to increase their cardiac index by 50%.

Inborn errors of metabolism, collectively as a group, form a relatively common indication for LT accounting for 9% and 26% of children under and over 2 years of age at the time of transplant, respectively. Metabolic diseases resulting in cirrhosis include alpha-1-antitrypsin deficiency, tyrosinemia, Wilson's disease, neonatal haemochromatosis, respiratory chain disorders, fatty acid oxidation defect and glycogen storage disease type IV, among many others. Metabolic diseases without structural liver disease include Crigler-Najjar syndrome type 1, glycogen storage disease type 1, propionic acidaemia, primary hyperoxaluria type 1, hereditary tyrosinemia, factor VII deficiency, ornithine transcarbamylase deficiency, familial hypercholesterolemia and protein C deficiency. Two series from the USA from the Scientific Registry of Transplant Recipients of 551 transplants [12] and Europe from King's College Hospital of 112 transplants reported excellent outcomes for this group [11]. Although the presence of cirrhosis did not appear to be a risk factor for worse outcomes, recipient black race, combined organ transplantation, ALF, hospitalization before transplant and age less than or equal to 1 year were predictors. The study from Sze et al. reported 11 ALTs with similar outcomes to whole liver replacement for noncirrhotic liver disease with an absent enzyme/gene product such as Crigler-Najjar type 1 [11].

Tumours

Liver transplantation for liver tumours in children accounts for 2–6% of all cases in European and American series. The most common indication is unresectable hepatoblastoma (following appropriate chemotherapy). Other tumours treated by LT include hepatocellular carcinoma, haemangioma, infantile haemangioendothelioma, rhabdomyosarcoma and epithelioid haemangioendothelioma. Angiosarcomas should not be transplanted as they invariably recur early. However, differentiation from more benign vascular tumours can be difficult even on histological examination of a biopsy. Clinical features such as pain, rapid deterioration or disease progression indicate sarcoma. The outcome of LT for unresectable hepatoblastoma is excellent with long-term patient and graft survival rates for cadaveric transplantation of 91%, 77.6%, and 77.6%, at 1, 5, and 10 years, respectively [13]. Patient and graft survival for children undergoing LDLT is 100%, 83.3%, and 83.3%, at 1, 5, and 10 years, respectively. Two North American series of 25 (HCC, 10 cases and hepatoblastoma, 15 cases) and 12 patients (HCC, 6 cases and hepatoblastoma, 6 cases) reported similar medium- and long-term survival rates for both tumours [14, 15]. Salvage transplantation for recurrent hepatoblastoma after conventional liver resection is less satisfactory with 5-year survival of 40% with a high rate of further recurrence. An analysis of

UNOS data of 336 patients with liver tumours which included 237 hepatoblastomas, 58 HCC and 35 haemangioendotheliomas noted that patient survival for the latter was inferior to that of hepatoblastoma (5-year survival of 72%) and rare liver tumours (5-year survival of 78.9%), but better than HCC (5-year survival of 53.5%) [16]. Tumour recurrence was the major cause of death in hepatoblastoma and HCC patients, but not haemangioendothelioma.

The development of hepatocellular carcinoma has been reported in BA, Alagille syndrome and progressive intrahepatic cholestasis, particularly BSEP (bile salt export pump) caused by a mutation in ACBC II gene. Children with tyrosinemia have a high risk of hepatocellular carcinoma before 2 years of age which appears to be markedly reduced by the use of 2-(2-nitro-4-3 trifluoromethylbenzoyl)-1,3- cyclohexanedione (NBTC) therapy [17]. For HCC, there are no criteria for selection comparable to the Milan criteria in adult patients. Macrovascular invasion of the portal vein trunk and first divisions continues to be a contraindication to transplantation due to the high risk of recurrence.

Acute Liver Failure

Acute liver failure (ALF) is defined by the onset of severe impairment of liver function in the absence of previous liver disease. Coagulopathy is always present, but in young children hepatic encephalopathy may be absent and is a late feature associated with a poor outcome. ALF is an indication for LT in 9% of under and 16% of over 2-year-olds in Europe and 15% of children in America. The cause of ALF cannot be determined in the majority of children (49% of all children and 54% of those aged 1 year) [18]. Potential causes include metabolic, paracetamol intoxication, autoimmune hepatitis, viral hepatitis, drugs, Wilson's disease, vascular disease and *Amanita phalloides* poisoning. The risk of death or liver transplantation is highest in children under 3 years of age. Logistic regression analysis has identified total serum bilirubin >5 mg/dL, INR >2.55 and hepatic encephalopathy as risk factors for death or liver transplantation. Of note, grade IV hepatic encephalopathy on admission was associated with higher rate of spontaneous recovery than those children who progressed to grade IV during the course of admission (50% vs 20%). Indications for LT are different from adults and an INR >4 (in the absence of disseminated intravascular coagulopathy) identifies the at-risk population.

Two recent series reported 5-year patient survival of 70% in children with ALF [19, 20]. Farmer et al. identified 4 factors which predicted graft or patient survival in 122 children with ALF which included cCrCl <60 mL/min/1.73 m (graft and patient), PELD >25 (graft), recipient age <24 months (graft) and time from onset of jaundice to encephalopathy <7 days (patient) [19]. The presence of 2 or more of these factors were associated a significant reduction in graft and patient survival to about 25–40%. Other series have also noted lower graft survival in children aged less than 2 years with ALF possibly reflecting technical challenges in small babies [21]. This population is the most challenging group and further improvements in perioperative surgical and intensive care are needed to make progress.

Acute liver failure in neonates is a rare but often fatal event characterized by a failure of synthetic function with coagulopathy. Hepatic encephalopathy is a late event and difficult to diagnose in infants [22]. Causes of ALF in neonates include metabolic, infectious and haematological disorders, congenital vascular/heart abnormalities and drugs. Congenital haemochromatosis is the commonest indication and the challenge is to provide a graft in time. Neonates and young children with ALF should only be treated in specialized paediatric hepatology centres with facilities for liver transplantation which continues to be the only therapeutic option with a long-term survival of over 60%.

Liver cell (hepatocyte) transplantation is an attractive option, as many patients only require short-term liver support while their own liver recovers. Human hepatocytes encapsulated in alginate beads (HMB) can perform the functions of the liver while alginate coating protects the cells from immune attack. Dhawan et al. reported their King's College Hospital experience and described the transition from animal models to human experience. Intraperitoneal transplantation of HMBs in healthy rats was safe and preserved synthetic and detoxification functions, without the need for immunosuppression. Subsequently, 8 children with ALF received HMBs (4 neonatal haemochromatosis, 2 viral infections and 2 children with unknown cause at time of infusion) at a median age of 14.5 days, range 1 day to 6 years. The procedure was well tolerated without complications. Of the eight children, four avoided LT while three were successfully bridged to LT following the intervention. HMBs retrieved after infusions (at the time of LT) were structurally intact, free of host cell adherence and contained viable hepatocytes with preserved functions. With HMBs 40 patients have been treated in total worldwide and 18 from the USA [23].

Timing of Transplantation

The timing of LT in children has been based on criteria established in adults and thus is focused on graft and patient survival. Optimal timing was viewed as listing for LT when expected survival was less than 18 months. Children with liver disease may not develop physically, intellectually and socially at a time of deteriorating liver function, and timing of transplant needs to take this into account. There is general agreement that Kasai porto-enterostomy should be performed for BA and that LT is reserved for those who develop progressive liver disease (apart from rare cases of late presentation >4 months).

MELD was introduced in 2002 as a response to increasing waiting list mortality for adult recipients. It provides a means of allocating livers based on likelihood of dying while on the waiting list. PELD was a similar mathematical tool based on data derived from the Studies of Pediatric Liver Transplantation (SPLIT) research group using bilirubin, INR, serum albumin, age >1 year and growth [24]. The introduction of MELD (and subsequently PELD) significantly decreased death or removal from the waiting list for being too sick within 2 years for both adults and children [25]. Cowles et al. [26] in reviewing a cohort of 71 children transplanted for BA (61, KP before LT; 10, primary LT) considered that PELD monitoring identified those in need of transplantation. Children with a PELD greater than 12 (n = 47) had a higher rate of post-LT mortality and retransplantation than those with a PELD of 10 or less. The authors suggested that a PELD score approaching 10 should trigger discussion of LT. PELD is the only scoring system currently used in children, and although helpful in advanced liver dysfunction, it is of limited value in the very young (under 1 year of age) and in older recipients, particularly with complications such as recurrent cholangitis, severe portal hypertension, pulmonary hypertension and hepatopulmonary syndrome [27–31]. Because of these limitations, PELD use has been largely restricted to North America. More research is needed to define optimal timing of transplantation in children to gain most benefit in terms of survival, growth and intellectual and social development.

Graft Allocation

Organs from deceased donors are mainly allocated depending on the blood group and organ size. Children will generally receive a liver from donors with the same blood group with certain exceptions. Children below 2 years old have received organs from mismatch blood group donors, but this is usually undertaken in circumstances when it is difficult to find a size match and good quality organ within a limited time such as in very sick patients or in countries where the only source of organs is live donors.

The size of the graft will also be important at the moment of organ allocation; it is generally difficult to have a whole size match organ for a small child; therefore, the main source of livers comes from adult donors, which are reduced or split to provide grafts that fit the children (generally left lateral segment (LLS)).

Graft Type

The type of graft used in children in need of an LT has also been in evolution. In order to match the graft-to-recipient body weight ratio, surgeons have developed techniques to resect segments of the liver from either a live or cadaveric donor. In case of a live donor, this requires estimating the required graft size and the liver remnant volume to ensure both donor and recipient have adequate function post surgery for it to be performed with compatible outcomes to cadaveric liver transplantation. For the youngest and smallest of the recipients, typically a LLS is utilized, while for a larger school-age child one may need the entire left lobe (LL). If the recipient is adult-sized, then a right lobe (RL) graft is required. For deceased adult donor, defined as suitable for routine use in children (<40 years of age, minimal fatty infiltration, ITU stay <5 days, satisfactory liver function tests), the liver can be split into two: extended RL and LLS, either in situ at the donor hospital or ex situ, after the liver has been retrieved on the back table. Long-term graft and patient survival has not been shown to be different between in situ or ex vivo procurement [32].

Criteria for "Split-able" Liver Grafts

Cadaveric donors who met the following criteria are considered to have "split-able" livers: (1) DBD donor <40 years of age, (2) intensive care unit (ICU) stay <5 days, (3) no low-dose vasopressor therapy, (4) estimated cold ischaemia time <10 hours, (5) ideal donor-to-recipient liver volume matching and (6) serum transaminase levels <3 times normal value. If only one of the criteria is missing and the others are well controlled, the graft can be accepted for splitting. However, despite these excellent outcomes, splitting of livers remain very limited with one recent study from the USA finding only 3.8% of the liver grafts that met the criteria for splitting were actually utilized. The number of "split-able" liver grafts was greater than paediatric waitlist deaths, and therefore this procedure has significant unrealized potential which could avoid mortality in young children.

The lack of age- and size-matched grafts and the underused "split-able" liver grafts are the reasons for high mortality in children. Expanding the criteria for SLT by increasing the upper age limit of the donors could help, but mandated splitting of younger donors remains the single most important way of providing sufficient grafts for young children. The lower age limit for "split-able" liver grafts has not been defined, but it is possible to split grafts from 15 kg donors. Gao et al. reported 16 children receiving split liver grafts from 8 paediatric donors <7 years with the youngest donor being 2.7 years old [33, 34].

Intraoperative

The first paediatric LT by Starzl and his team would not have been possible but for the prior experimental preclinical work using large animal models. Once established, however, this

was followed quickly by technological innovations including the development of organ donation and implantation surgical techniques based on the ability to preserve livers in cold storage solutions.

Whole Liver Transplantation

Whole liver replacement is relatively uncommon in children under 5 years of age at the present time. Above the age of 5 years, it becomes more common. The transplant involves excision of the diseased liver, by division of the common bile duct (or Roux loop if there has been previous biliary surgery), hepatic artery, portal vein and IVC above and below the liver. Orthotopic liver replacement is accomplished by anastomosis of the corresponding structures with the donor liver and achieving haemostasis; the alternative technique is the piggy-back technique whereby the donor's suprahepatic IVC is anastomosed to a common orifice of the hepatic veins with the native IVC kept intact. Management of intraoperative coagulopathy is an essential component of the operation. The technique is very similar to adult LT, but the smaller size of the vascular structures demand a more refined surgical technique, especially for arterial reconstruction. The use of optical magnification or the operating microscope appears to reduce the incidence of postoperative vascular and bleeding complications. The use of cell salvage has led to bloodless surgery becoming a practical proposition. Closure of the abdomen should only be performed if there is no risk of graft compression.

Partial Liver Grafts

The use of partial grafts was the solution to both organ shortage due to size mismatch in children. More than 10,000 LT have been performed in Europe in recipients under 16 years old. Of these, approximately 40% have been performed with whole organs. Partial grafts account for 80% and 52% of all LT performed among patients aged 0–2 and 2–15 years old, respectively. Early experience with reduced size grafts, either left lobe or left lateral segment, then led onto to split and living donor LT with both techniques being incorporated into routine clinical practice from 1991 onwards. Roberts et al. analysed data of 6467 LTs performed in patients under 30 years old from the SRTR-OPTN database [35]. It was noted that patient and graft survival during the first year after transplant for each donor graft type varied according to the recipient age group. For children of 2 years and under, living donor grafts had a 51% and 30% lower relative risk (RR) of graft failure than deceased donor split (DD-S) and deceased donor full (DD-F), respectively. A similar difference in mortality risk in the same group of age favoured recipients of LD grafts over DD-S (RR = 0.71, $p = 0.08$). Recipients in the 0–2 year age group had higher risk of mortality and graft failure with DD-S livers than DD-F livers (RR = 1.31, $p = 0.04$ for mortality; RR = 1.42, $p < 0.001$ for graft failure). For patients aged 2–10 years, the relative risks of mortality and graft loss were higher after LD than after DD-F (RR = 1.78, $p = 0.02$ for mortality; RR = 1.53, $p = 0.02$ for graft loss) but not after DD-S. In the 11–16 years age group, a significantly higher relative risk of graft failure was observed after LD than after DD-F transplant (RR = 3.63, $p = 0.0001$) or DD-S transplant (RR = 2.87, $p = 0.02$), although mortality risks were similar for all three donor graft types. Subsequent publications have confirmed the excellent outcomes with living donor LT in young children, but published experience with children over 12 years of age remains limited outside of India and the Far East.

Publications of outcomes of LLS reduced size LT tend to be from the early 1990s, and there are few direct comparisons with those observed after split LT (SLT). SLT has become an established technique to transplant children while preserving the pool of liver grafts for adults. A recent study of 251 LTs which included 138 reduced and 30 split reported 1-year patient and graft survivals that were comparable at 73% and 67%, respectively [36]. In addition, no differences in vascular complications were observed. Of note, biliary complications were significantly more common after split when compared with reduced-size grafts (21% vs 4%, $P < 0.0001$). The most common biliary complication after SLT was late stricture, in contrast to RLT, which was cut surface bile leak. Patients undergoing SLT had a 6.7-fold increased risk of biliary complications compared to those receiving a RLT; however, these complications did not appear to impact on long-term graft or patient survival. A further series from UCLA has compared the outcome of whole and partial grafts in both adult and paediatric recipients [36]. Of 442 LTs, 284 were whole, 109 were split left lateral segment (SL-LLS) and 49 living donor left lateral segment (LD-LLS). The 10-year patient survival was similar for all graft types. Multivariate analysis confirmed that history of previous LT and SL-LLS were independent predictors of reduced survival. Chronic rejection and hepatic artery thrombosis were the most common reasons for graft loss in these children. Hong et al. reported outcomes after partial liver grafting in children analysing data from the SPLIT registry and compared the outcome of each graft variant with that of whole organ transplantation (1183 whole, 261 split, 388 reduced and 360 live donor (LDLT)) [37]. There was a clear difference in outcomes at 1 year (W: 93%; R: 82%; S: 87%; L: 89%) and 4 years (WLT 89%, RLT 79%, SLT 85%, LDLT 85%) post-transplant. However, the groups were not strictly comparable in terms of era of transplant, recipient selection and centre. Children receiving a technical variant waited on average 2.3 months less than those receiving a

whole liver and tended to be younger. Complications were significantly higher after partial grafts: at 24 months the incidence of biliary complications were WLT 17.3%, SLT 28.5%, RLT 25.3% and LDLT 40.1% and vascular complications were WLT 16.5%, SLT 23.8%, RLT 23.5% and LDLT 24.4% [38].

Trying to understand and balance the increased opportunity of being transplanted against the higher incidence of early complications associated with SLT can be difficult. Merion et al. tried to address this issue by comparing the predicted lifetimes for SLT for an adult and child recipient versus WLT for an adult to try to determine the best use of this limited supply of organs [39]. They analysed mortality risk for 48,888 patients on the waiting list, 907 SLT and 21,913 WLT recipients (between 1995 and 2002). Of 23,996 donor livers used for transplantation, 533 grafts were split. Donors aged 10–39 years accounted for 81.6% of split livers and 48.5% of livers used for WLT. Only 11.8% of livers that were split were from donors older than 40 years. They analysed years gained per SLT performed against the waiting list death ratio and concluded that "the potential annual net gain in life years could be as high as 169 patient-years in the first 2 post-transplant years, if all livers meeting accepted criteria were used for splitting". For every 100 donor livers, an extra 11 years of life was predicted over the first 2 years of follow-up if organs were split rather than transplanted whole. In addition they identified a significant survival benefit for paediatric recipients of SLT compared with children continuing on the waiting list and concluded that enough grafts could be provided to satisfy the entire demand for paediatric donor liver grafts.

Living Donor Liver Transplantation

Living donor liver transplantation (LDLT) has become an important source of grafts for children worldwide. Following on from the first description by Raia et al. in 1988 [3], the first long-term survivor from Strong et al. in 1989 [40] and the early series published by Broelsch et al. [41], the technique has become established with excellent short- and long-term survival. Today LDLT is the norm in parts of the world (such India and the Far East) where religious or cultural sentiments or medical infrastructure necessitates this option in response to the lack of robust cadaveric organ-sharing systems, and multiple strong living donor programmes have been established. At present LDLT contributes to 10–20% of LTs performed in the USA and Europe. The work-up time for LDLT varies between 2 days and 3 weeks depending on the urgency of the procedure and the centre volume and experience.

The LLS is the most commonly used graft [42]. Full left or right lobe grafts are used less commonly and tend to be used in young adults. Donation is most commonly from parents although other family members and altruistic donation have been reported. The ethics and understanding of the desire to donate for children are easily understood and the risks appear to be low, but not negligible. Donor mortality for LSS grafts is of the order of 1:1500 or less. The incidence of other significant donor complications which include bile leak (1–2%), bleeding and the need for transfusion (1–2%), deep vein thrombosis and pulmonary embolus (1:1500?) and incisional hernia (5–7%) are considered "acceptable". Risks of donor mortality are similar for left hepatectomy but rise to 0.3% for right lobe donation. The risks of bile leak are also higher for the donor, particularly with the right lobe grafts where the incidence may be as high as 5%. Donor age above 55 years is generally regarded as a contraindication (especially for the right lobe) due to the slower regeneration and the increased risk in this population.

The LLS segment graft accounts for approximately 20–25% of the adult liver but provides a full sized "liver" for the child. Assessment of the donor is designed to ensure that the liver segment is anatomically and functionally suitable for transplantation and to identify additional risk factors such as procoagulant abnormalities, donor smoking and steatosis within the liver. Many centres continue to follow the two-stage assessment and consent model reported by Brolesch et al. which includes psychiatric/psychological assessment of the donor and family with a 2-week cooling off interval before completion of the consent process [41]. Depending on the availability of cadaveric transplantation, suitability for living donation will vary from 50 to 90% when assessing families. The techniques of surgery have been standardized and outcomes are excellent for all liver diseases. The advantages of LRLT include the ability to perform surgery electively with a good quality graft, dry cut surface (minimal blood loss) and excellent outcome. The incidence of early rejection is similar to that of cadaveric transplantation, but in the long term, it is considered, although the supporting evidence is limited, that more of these recipients may be tolerant of their grafts and that immunosuppression may be more likely to be weaned or withdrawn. Lifelong calcineurin inhibitor exposure is considered to be considerably lower.

Morioka et al. reported the long-term outcome of LRLT for metabolic disorders in 46 children [43]. Mean age at diagnosis and LDLT was 48.6 (0–196) and 86.5 (1.4–199) months, respectively, with survival rates of 86.9% and 81.2% at 1 year and 5 years post-transplant. Patient survival was significantly better in children with liver-centred disease which included Wilson disease, ornithine transcarbamylase deficiency, tyrosinemia type 1, Crigler-Najjar syndrome type 1 and bile acid synthetic defect than in those with non-liver-centred disease (glycogen storage disease, propionic acidaemia, methylmalonic acidaemia and erythropoietic protoporphyria) ($P = 0.003$). Further statistical analysis

showed that cumulative patient survival of patients with normal or slightly delayed physical growth at the time of LDLT were significantly better than those with delayed physical growth ($P = 0.012$).

LDLT is an excellent option in the management of ALF in children. The work-up can be performed rapidly if the unit is regularly performing elective LDLT. Ethical concern has been expressed regarding the ability of the donor to appreciate the risk in these circumstances [44]. However the lower risk of LLS donation has led to the use of LDLT as an accepted therapy for children with acute liver failure. Greater debate surrounds the use of right lobe living donation for ALF particularly in countries with effective cadaveric organ donation.

Recent Developments in Transplant Surgery

Over the past 30 years, surgical techniques have become standardized worldwide. Left lateral segment and left and right LT are used to overcome size discrepancy and to engraft the majority of children. Split and living donor LT have been key to sustaining the reduction in deaths on the paediatric waiting list. Auxiliary LT offers scope for native liver regeneration in children presenting with ALF who are able to withdraw from immunosuppression in the majority. Hepatocyte transplants remain experimental and have been used to bridge young children with metabolic liver disease through to transplantation. The current challenge is to minimize technical complications that impact on graft survival, such as hepatic artery thrombosis and biliary strictures, and continue to improve outcomes. New technologies are emerging that are currently under evaluation and will impact on practice of which machine perfusion is perhaps the most promising.

Auxiliary Liver Transplantation

Auxiliary liver transplant (ALT) was first described experimentally by Welch in 1955 [45]. The auxiliary liver was placed in a heterotopic position in the right paravertebral gutter, with portal venous inflow from the iliac vein. The idea of heterotopic ALT was attractive as it avoided the need for native hepatectomy with the aim of improving hemodynamic stability during surgery. The first experience of ALT in a human was reported in 1964, using a heterotopic graft, with the idea of avoiding difficulties presented by whole liver replacement. Chenard-Neu et al. reported long-term survival of 2 out of 47 patients who underwent ALT between 1964 and 1980 [46]. From 1986 onwards, outcomes of ALT began to improve, although only small numbers were performed [47]. The technique of ALT proved to be more difficult than that of LT with a higher rate of technical complications and inferior graft function and outcome. The development of hepatocellular carcinoma in the cirrhotic liver remnant of one long-term survivor led to the abandonment of ALT as a treatment for chronic liver disease.

The technique has become an established indication, however, for acute liver failure especially in children. In addition ALT has also been used to treat noncirrhotic inborn errors of metabolism based in the liver [43], although this has not been accepted as a standard treatment. For ALF the aim is to treat children satisfying established criteria for transplantation, with survival equivalent to that obtained with whole liver replacement with subsequent native liver regeneration and immunosuppression withdrawal. Children have the most to gain from avoiding the complications of lifelong immunosuppression. An early multicentre European experience identified that recipients under 40 years of age and particularly children were most likely to survive, have successful liver regeneration and withdraw from immunosuppression [47]. In addition, patients with hyperacute liver failure were more likely to regenerate than those with subacute liver failure and that orthotopic rather than heterotopic ALT had a better outcome. Immunosuppression withdrawal has been possible in more than 70% of survivors [48–51].

ALT have included the use of whole liver, right lobe, left lobe or left lateral segment grafts implanted into an orthotopic position. A proportion of the native liver is resected to make room for the graft. The graft is piggy-backed onto the cava, portal inflow is established by end to side anastomosis, and arterial inflow is established using either a donor iliac conduit or to a branch of the native hepatic artery. Biliary drainage is achieved with either a short Roux-en-Y hepaticojejunostomy or by anastomosis to the native hepatic bile duct. In children the majority of ALTs are performed using left lateral segment grafts to overcome donor-recipient size discrepancy. Selection criteria include the recipient satisfying existing criteria for LT for ALF, with no neurological or cardiovascular contraindication and a suitable graft. The donor liver should be of excellent quality to ensure that good early function is achieved. Marginal livers are difficult to use as partial grafts and provide inferior function and should be avoided.

Postoperatively patients are managed with conventional immunosuppression. Early graft dysfunction is due to technical complications, particularly inadequate venous inflow or outflow, and poor quality or small-for-size grafts. The serum AST and INR may be slower to settle than with conventional transplantation. Persistent elevation of the serum bilirubin indicates complications with the graft. Postoperative bleeding with the need for relaparotomy is more common due to the presence of two cut surfaces.

Patients are followed with CT or MR imaging and guided biopsies of both graft and native livers in the early postoperative period and at 3-month intervals to assess liver recovery. Dimethyl iminodiacetic acid (HIDA) scintigraphy is also of

value in assessing the differential function of the two livers and documenting native liver recovery. The decision to begin immunosuppression withdrawal is usually made at 6 months or when signs of regeneration are observed on biopsy. Gradual weaning is necessary to avoid severe graft rejection and infarction. The graft will usually atrophy and disappear without the need for resection. Immunosuppression withdrawal may need to be started to stimulate native liver regrowth. Even after massive hepatic necrosis, the liver regenerates fully. However, larger the graft transplanted, slower the regeneration of the native liver is, without a reduction in the level of immunosuppression. In the subacute group, regeneration is less rapid, as observed by sequential imaging. Successful immunosuppression withdrawal has been reported up to 4 years after APOLT.

Histopathological assessment of the native liver at the time of ALT has identified patterns of hepatocyte cell loss that provide insight into the likelihood of regeneration. A diffuse pattern with uniform cell loss throughout was associated with hyperacute liver failure and excellent regeneration with restoration of normal histology in over 70% of patients [52]. A map-like pattern was associated with seronegative hepatitis and in adult patients had a mixed outcome, in contrast to children where the results were excellent. In a small number of cases, no viable hepatocytes could be identified, and this group did not regenerate effectively. Of 5 cases in our series of over 60 adults and children cases with this pattern, 3 died and 2 were retransplanted. ALT should be considered the gold standard for the treatment of children with ALF requiring transplantation.

DCD Donation

Great efforts have been made by the transplant community to expand the donor pool for paediatric LT with the selective use of DCD grafts. In the last few years, an increasing number of DCD grafts have been transplanted into children, with good graft and patient outcomes in the medium and long terms with low incidence of morbidity. The majority of DCD have been from controlled donation (within the hospital intensive care environment) and usually have unrecoverable brain injury. Withdrawal of support occurs and death is pronounced clinically and with ECG. There follows a 5 minute standoff before surgical retrieval is initiated. Warm ischaemia is calculated from the onset of systolic BP <50 mmHg or PaO_2 <70% to cold perfusion of the liver. The organs have been used successfully in children, either as whole or reduced sized grafts. Selection criteria for liver reduction include donor age <40 years, warm ischaemic time of <30 minutes, good liver function and no steatosis with the cold ischaemic time being kept under 8 hours. Several centres including our own have reported RLT DCD paediatric transplants with excellent graft and patient survival with a low incidence of biliary complications such as cholangiopathy [53]. Careful donor selection and short donor WIT and CIT appear to be important for good outcomes. The use of DCD liver grafts in children could potentially be increased by the use of in situ regional normothermic machine perfusion to optimize graft quality. Moreover graft preservation on machine perfusion could permit assessment of organ viability and reduce the impact of ischaemia-reperfusion injury. The new frontier is the technical feasibility of splitting livers during NMP which could increase the number of partial grafts available for children and change the logistics of splitting DCD livers.

Small Infants

Yamamoto et al. reported outcomes despite the surgical challenges of LT in infants 3 months old or younger in a single institution and compared the results with infants 3–6 months old. Acute liver failure was the main indication for LT in the infants under 3 months of age group (XS group $n = 31$, 84%) versus 3–6 months (S group $n = 7$, 26%). The overall incidence of hepatic artery thrombosis and portal vein thrombosis/stricture were 5.4% and 10.8% in the XS group and 7.4% and 11.1% in the S group, respectively (not significant). The overall incidence of biliary stricture and leakage were 5.4% and 2.7% in the XS group and 3.7% and 3.7% in the S group, respectively (not significant). The 1-, 5- and 10-year patient survival rates were 70.3%, 70.3% and 70.3% in the XS group compared with 92.6%, 88.9% and 88.9% in the S group, respectively [54].

On a technical level, the major concern for LT in small infants is the shortage of size-matched or split/reduced livers which contributes to the high waitlist mortality reported in the neonatal age group. The development of technical variants of hyper-reduced or monosegment grafts has proved to be important in ensuring these children are transplanted and has reduced the waiting time and waitlist mortality.

Although the left lateral section (LLS) is used for the paediatric population, this graft may be too large for small infants. Large-for-size grafts can be problematic with microcirculatory hypoperfusion due to a combination of low portal blood flow and external compression of the liver by the small size and depth of the abdominal cavity. The option of monosegment grafts can overcome large-for-size problems. When deciding whether monosegment reduction is necessary, the relationship between the shape (thickness and length), volume, conformation of the graft and the size of the abdominal cavity has to be taken into consideration. For many centres, a GRWR >4.0% combined with a ratio of graft thickness and abdominal depth can be a guide to when reduction is indicated. Reports of monosegments have described the use of a nonanatomical reduced LLS grafts with either segment 3 or segment 2 has shown excellent outcomes [55].

Another option to prevent large-for-size complications is delayed abdominal closure using a silastic mesh or skin closure to avoid graft compression and have no adverse effect on graft/patient survival [56].

The use of ABO-incompatible grafts has been another way to increase graft options for small infants. Both paediatric and adult ABO-incompatible LT survival has improved markedly and has become comparable to ABO-compatible LT on the introduction of rituximab prophylaxis before LT. However, infants under 1 year of age are able to accept ABO-incompatible grafts without desensitization therapy because the immune system is still developing. This can be confirmed by measuring the levels of anti-A and anti-B antibody titres (<1/8) [57].

Post-transplant

Postoperative Immunosuppression

Over the 60 years, paediatric LT has seen serial improvements in morbidity and mortality. These improvements are related to immunosuppression (IS), donor selection and maintenance, surgical technique and complex team working. The focus of IS has been on the prevention of acute rejection and graft loss; however, in children, morbidity and mortality rates from infections exceed those from rejection and can also impair growth and renal function and increase the risk of some cancers. Getting the balance of IS right in the short and long term is key to a successful LT programme. Evidence is also accumulating that children may be more likely to develop graft tolerance and to wean from IS in the long term [58], particularly those with biliary atresia and with the use of LD grafts.

Early post-transplant IS has been the increasing use of induction therapy (26%), with IL-2 receptor antibodies as renal sparing regimen. For maintenance IS, calcineurin inhibitors (CNI) remain the cornerstone, but cyclosporin has been largely replaced by tacrolimus. Children often receive an antiproliferative agent such as azathioprine or mycophenolate mofetil to supplement IS or for CNI/renal sparing. Tacrolimus trough levels of 8–10 ng/l in the first 3 months and 5–8 ng/l thereafter are usually sufficient. In the longer term, tacrolimus levels can be further weaned to low levels, particularly if graft function is normal with no rejection episodes. Moderate to severe acute rejection is treated with steroid boluses, and if unresponsive, antithymocyte globulin can be given.

Over-IS is associated with long-term side effects including renal impairment, hypertension, lymphoproliferative disease and cancer and may hinder the process of immune engagement and the development of immune tolerance. There has been a move towards progressive minimization of IS, including steroid withdrawal which is associated with a growth advantage without any significant rejection-related complications [59, 60]. Many units discontinue steroids during the first or second post-transplant year, and more than 50% of children are on monotherapy tacrolimus by 18 months post-LT [61]. It has been suggested that early steroid withdrawal may be associated with a higher risk of acute and chronic rejection and development of de novo autoimmune hepatitis (AIH) [62]. The pathogenic mechanisms behind de novo AIH remain unclear, but it appears to be related to under immunosuppression and affects 2–5% of paediatric LT recipients [63, 64].

Calcineurin inhibitors share a number of side effects in common including nephrotoxicity, neurotoxicity and hyperlipidaemia [65]. There are, however, some differences between tacrolimus and cyclosporin. Cyclosporin is associated with hirsutism and gum hyperplasia, while tacrolimus is associated with diabetes and hair loss. For teenagers, especially in females, tacrolimus is often the CNI of choice because of the lower incidence of gum hyperplasia and hirsutism. Tacrolimus has greater water solubility and less dependence on bile salt absorption, thus resulting in improved bioavailability over cyclosporin. However, it has larger inter-individual variations. The introduction of once daily preparations of CNI may help reduce variations in drug levels and improve adherence to medication [66]. Tacrolimus may be superior to CsA with regard to steroid withdrawal and the incidence of acute and chronic graft rejection. CNI sparing or substitution with mTOR inhibitors such as sirolimus or everolimus are used for patients with nephrotoxicity, but their efficacy requires validation in long-term studies in large cohorts.

In children surviving more than 1 year post-LT, once daily CNI formulations, particularly taken in the morning, also improve adherence without increasing the incidence of acute rejection, graft loss or death [66]. Non-adherence is one of the commonest causes of late mortality and is the leading cause in adolescents and young adults [67]. Non-adherence has been linked to a number of factors including traumatic stress disorder from the time of transplant, a lack of knowledge and understanding of their medical history and an absence of continuing medical education. Psychological intervention and ongoing education can provide effective intervention and reduce the likelihood of graft and patient loss.

Surgery-Related Complications

Early surgical post-LT complications are primarily related to the vascular and biliary reconstructions. Outcomes and technical complications after paediatric LT are influenced by transplant volume and available expertise. It has been reported that 1-year patient death ratios were significantly lower for high-volume centres (>16 procedures per year) (0.77) than for low-volume centres (<7 procedures per year) (1.23, $P = 0.027$). Colocation of adult and paediatric liver transplantation may also contribute to better outcomes [68].

Primary Graft Non- or Dysfunction

Early failure of the graft is relatively rare (1–2%), and retransplantation is the only lifesaving therapy. Underlying causes of primary nonfunction may be due the mode of death of the donor, problems with retrieval, preservation or implantation. Signs of poor graft function include haemodynamic instability requiring continuing inotrope support, persistent or increasing acidosis and lactataemia and bleeding due to persistent coagulopathy. INR greater than 4 and first day AST levels of greater than 2000 IU/l reflect major cell damage, but even levels of more than 5000 IU/l may settle with good long-term graft function. Early high liver AST levels are seen with DCD donors without impacting on outcome and these settle rapidly after the second postoperative day.

Bleeding

Postoperative intra-abdominal haemorrhage is a common complication in the first 48 hours after transplant occurring in 5–10% of patients. Risk factors include renal failure, perioperative dialysis and acute liver failure. Re-exploration may be necessary, but a definite bleeding point is identified in only half the cases. Bleeding from coagulopathy associated with graft dysfunction needs to be resolved rapidly to avoid hypotension and further graft injury.

Caval Complications

The reported incidence of hepatic vein-inferior vena cava anastomotic complications is low and occurs either as a result of technical failure or as a consequence of graft hypertrophy and remodelling. Caval complications are rare in size matched and full left liver graft with caval replacement LT with an estimated incidence of less than 1%. The use of interrupted anterior sutures in all venous anastomoses may help to avoid stenosis and kinking in the short term. The incidence of hepatic venous obstruction is higher after left lateral segment LT and is of the order of 2–4% and dependent on technique. It is always considered to be hepatic veins outflow associated with caval stenosis at the inferior aspect of the anastomosis or with twisting. It is a risk at retransplantation particularly if the native cava is small. The most important technical development was the triangulation technique for LHV/caval anastomosis which significantly reduced the incidence of complications. Fixing the graft at the end of the procedure also helps to avoid rotation of the liver and kinking of the hepatic vein anastomosis. Clinical manifestations of venous outflow obstruction include hepatomegaly, persistent ascites, unexplained renal impairment and peripheral oedema (if there is caval stenosis) [69]. It may start insidiously two or more weeks after the transplant. The characteristic features of venous outflow can usually be seen on CT angiography and confirmed by pressure measurements across the cava and anastomosis. Balloon angioplasty is usually effective, but occasional hepatic venous and/or caval stents may need to be inserted [70]. Long-term patency rates have not been reported and long-term anticoagulation may help prevent further complications.

Portal Vein Thrombosis and Stenosis

Portal vein thrombosis (PVT) and stenosis occur in up to 4% of LT, with a higher incidence after LLS LT particularly for biliary atresia and when the PV is small or with significant extrahepatic venous collaterals. Early PVT may be asymptomatic for the first day or two but develop gastrointestinal bleeding from the jejuno-jejunal anastomosis, rising INR and increasing ascites (occasionally chylous) if there are few extrahepatic collaterals. If it extends into the liver, hepatic dysfunction may become evident, with rising transaminases which may progress to liver failure. If the extrahepatic PV is involved, collaterals may prevent the development of severe portal hypertension, and normalization of liver function may occur. With clinical stabilization, the INR does not fall below 1.4, the platelet count is low and signs of portal hypertension persist. Risk factors for PVT include discrepancy in calibre between donor and recipient PV (atrophic or pre-duodenal PV associated with biliary atresia), the length of PV, twisting, technical failure and graft rotation [71]. Doppler ultrasound and/or CT angiography will identify the problem. Early diagnosis and surgical correction will rescue the situation. Care must be taken to remove all the clot in the native PV and SMV, and any significant collaterals must be ligated to ensure there is good forward flow in the PV. If PVT is recognized late, when collateralization has developed and liver function is normal, then intervention can be avoided. If there are late complications from persistent portal hypertension, then a Rex or portocaval shunt should be attempted again with ligation of the venous collaterals. Liver biopsy should be performed prior to surgery to identify any changes such as nodular regenerative hyperplasia which may influence the surgical decision. An analysis of 521 paediatric LDLTs identified PV thrombosis or stenosis in 9% with 6 graft losses secondary to early complications [71]. The incidence of PV complications within 3 months of LT was 1.7% and body weight of less than 6 kg was the main risk factor on multivariate analysis. Early recognition and surgical correction was key to avoid graft loss. The platelet count is a valuable indicator of the presence of portal hypertension and the presence of late PV complications.

Porto-mesenteric venous occlusion is a relative contraindication to LT. If there is a large varix or an isolated vein, then a jump graft may be sufficient to revascularize the liver. Many of these patients are young adults with the JAK-2 mutation and the risk of further thrombosis is high and coagulation post-transplant is essential. JAK-2 mutation is a rare cause of venous thrombosis in young children. Portocaval hemitransposition has been utilized in predominantly adult recipients for extensive porto-mesenteric thrombosis; how-

ever, the incidence of primary graft nonfunction and retransplantation are high. Survival of 62% has been reported in 34 cases with relatively modest long-term follow-up [72]. The use of the left renal vein particularly in the presence of a lieno-renal (collateral) shunt has become the preferred way of portal venous revascularization in this difficult group of patients with improved long-term survival.

Arterial Complications

Hepatic artery stenosis (HAS) and thrombosis (HAT) occur in up to 14% and 7% of paediatric LTs, respectively [73], with a mean incidence of 8.3%. A higher incidence of HAT after adult LT has been reported from low-volume centres (5.8% with <30 LT cases/year versus 3.2% with >30 LT cases/year). No data are available for the performance of individual surgeons with respect to the number of LT procedures performed or centre activity.

Early HAT is silent and daily ultrasound for the first 5 days offers the best opportunity for early revascularization and graft salvage in children. HAT should be suspected if there is a fever or a positive blood culture for a gram-negative organism in the first month after LT [74]. If unrecognized, then the subsequent clinical course will depend on the potential for and the efficiency of developing a collateral arterial circulation and supervening infection within the compromised biliary tree. Liver dysfunction may occur with modest transaminitis and subsequent cholestasis. The clinical picture can mimic early rejection. With the development of parenchymal ischaemia, the transaminitis becomes more pronounced. Increasing cholestasis and the onset of cholangitis mark the development of significant biliary ischaemia and cholangiolitic abscesses with associated parenchymal necrosis. Untreated, they can progress to liver failure and death. A more protracted course of recurrent cholangitis and chronic ill health may ensue. The pathognomonic sign of HAT is the development of a nonanastomotic/complex biliary stricture, most commonly at the hilum. The ischaemic biliary tree sheds damaged biliary epithelium, and densely adherent casts form on the ulcerated surfaces. These casts and duct ischaemia predispose the patient to recurrent cholangitis and obstructions with the development of biliary abscesses and liver infarction.

Factors influencing collateralization are poorly understood but include the site of the arterial thrombosis (the closer it is to the hilum, the more likely), the graft type (split/reduced grafts may be more likely to collateralize than whole livers), Roux-en-Y hepatico-jejunostomy, multiple arteries and the timing after LT (patients with a later occurrence are more likely to survive). The overall mortality rate for patients with early HAT has been estimated to be as high as 33%. Just more than half of the recipients with early HAT lose their grafts [75]. The clinical burden of retransplantation and the use of precious grafts to salvage these patients is high and is mirrored by the escalating financial costs.

The surgical causes of early HAT include retrieval injuries (e.g. intimal tears, dissection and hematoma), technical problems with anastomotic stenosis or kinking and small or multiple arteries requiring arterial reconstruction and use of arterial conduits [76]. Other reported risk factors have included split liver grafts, DCD and neonatal donors, CMV-negative recipient, long cold ischaemia, large liver (graft-to-recipient body weight ratio >3–4%), small-for-size liver (graft-to-recipient body weight ratio <0.8%) and ABO incompatibility. Retransplantation is also associated with a higher incidence of HAT from both surgical and nonsurgical complications. Nonsurgical factors contributing to HAT include procoagulant states such as Janus kinase 2, anticardiolipin antibodies, factor V Leiden deficiency and a high haematocrit during the early postoperative course. Procoagulant states may also be associated with particular liver diseases such as autoimmune disease and sclerosing cholangitis, high ascitic drain loss and use of drugs such as aprotinin and tranexamic acid. Vessel fragility with associated alpha-1-antitrypsin deficiency and primary hypercholesterolemia also increase the risk of HAT.

Interventions for early HAT include urgent revascularization with thrombectomy, vascular anastomosis revision and thrombolytic drug therapy. Traditionally, the choice was urgent retransplantation or conservative management. In a comprehensive review of HAT, Bekker et al. looked at outcomes after various interventions in adults and children [73]. Revascularization was attempted in 54% of children with an overall success rate of 56%. A correlation was noted between early intervention and successful outcome. Daily ultrasound examinations for the first week were associated with early recognition and better outcomes (66% versus 45%). Children were more likely than adults to have a successful outcome after early revascularization (61% of adults and 92% of children). Retransplantation was performed for rescue in up to 62% of children with a mortality rate of at least 30%.

Early hepatic artery stenosis (HAS) has also been recognized as a predisposing risk for both HAT and graft ischaemia/loss. Risk factors include clamp injury, intimal trauma caused by perfusion catheters, technical, intimal hyperplasia and severe rejection. In a recent study by Sommerdale et al. in adults, of 37 patients treated by transluminal radiological intervention, hepatic artery patency was 94.6% with 5-year graft and patient survival rates of 82% and 87%, respectively, at median follow-up of 66 months [77] . Repeat interventions were helpful in 20% of treated recipients with an overall incidence of HAT of 11%. The use of Doppler ultrasound to identify HAS with a tardus parvus waveform (defined as a waveform with a resistive index <0.5 and a systolic acceleration time <0.08 seconds) has been associated with a low positive predictive value and a high false-positive rate [78].

When combined with an optimal peak systolic velocity of 48 cm/second, it has an improved specificity of 99% and a positive predictive rate of 88% with a false-positive rate of 1%. The early identification of tardus parvus with appropriate interventions may reduce the incidence of subsequent thrombosis. Further data is needed for children with HAS.

The management of early HAT is based on the appearance of the liver on CT angiography which will confirm thrombosis and reveal the presence of parenchymal ischaemia. If the serum transaminases are normal and there is no parenchymal ischaemia, then revascularization should be attempted with or without accompanying thrombolysis [79]. If there is significant transaminitis or definite parenchymal ischaemia on CT, then revascularization may produce a significant reperfusion injury that may endanger the child. Late recognition should be managed conservatively with rescue retransplantation for those developing ischaemic complications. The routine performance of retransplantation for all cases of early HAT is not indicated in an era of organ shortages.

Biliary Complications

Biliary complications remain a significant problem after LT in children with an incidence of 11–38% [80]. Factors associated with biliary complications include anatomical variations of biliary anatomy, ischaemia, technical failure, DCD grafts, ABO incompatibility and cytomegalovirus (CMV) infection. The commonest early complication is biliary leak which may be anastomotic or nonanastomotic. Anastomotic leaks are usually due to technical failure, but aberrant right posterior sectoral ducts may be missed. Non-anastomotic leaks are only seen with segmental grafts and usually represent missed or anomalous segment IV or segment I ducts. Strictures may be early or late and occur at both anastomotic and nonanastomotic sites [80]. Most anastomotic strictures are secondary to technical failure or scar tissue causing retraction and narrowing of the CBD at the suture site, although covert ischaemia may also be a factor. Anastomotic strictures will require surgical or radiologic intervention. Strictures occurring within the first year after LT will usually respond to percutaneous dilatation. Nonanastomotic strictures are probably caused by bile duct ischaemia due to arterial insufficiency. This ischaemic phenomenon is responsible for the loss of the biliary epithelium, producing multiple focal areas of intrahepatic biliary duct strictures separated by dilatations. These strictures may occur anywhere in the biliary tree and if extensive inevitably lead to retransplantation. A recent report of 126 LT in 108 children identified biliary complications in 30 cases, including leak (14), stricture (14), necrosis (9) and biliary occlusion caused by a drain (1) [80]. 56% of these with complications required surgery. Serum GGT peak value in the first week (358.8 ± 283.7 vs 251.3 ± 194 U/L) was considered a good early non-invasive marker to identify recipients at risk of biliary complications. In contrast, Anderson et al. reported 66 LT, of whom 17 (26%) developed biliary complications that required intervention [81]. The stricture rate was 16% for whole, 27% for split and 43% for LDLT and none after reduced sized grafts. Sixteen patients were treated with radiological percutaneous biliary dilatation, and one was treated with endoscopic stenting with complete resolution in 12 (71%). The remainder underwent surgical correction. Nonanastomotic or diffuse biliary strictures are associated with hepatic artery thrombosis, DCD grafts, preservation injury and transplantation with ABO-incompatible grafts and invariably come to retransplantation.

Other Surgical Complications

Bowel perforation is a rare complication except in children with biliary atresia who have undergone previous surgery when the incidence can be as high as 15%. Division of adhesions, portal hypertension, use of diathermy, cytomegalovirus enteritis, sepsis, use of inotropes, steroids and malnutrition have all been implicated. Paralysis of the right diaphragm due to a phrenic nerve injury at surgery may occasionally be the cause of failure to wean from the ventilator despite excellent gas exchange, and a "normal" chest x-ray and is a particular problem after retransplantation in small babies. Diaphragmatic hernia has been reported in up to 2% of LTs and has been associated with use of LLS grafts, young children, ascites and large-for-size grafts. The hernias may contain small and large bowel and should be repaired promptly to avoid ischaemic complications. Children with a combination of chest and abdominal symptoms should have imaging to exclude diaphragmatic hernia.

Retransplantation

Retransplantation is performed in up to 15% of children, although the incidence has falling consistently over 30 years. Hepatic artery thrombosis accounts for 50% of cases, and patient survival of 82% has been reported with elective versus 46% for emergency retransplantation. Retransplantation for primary nonfunction or in the setting of multi-organ failure has a survival of less than 30%. However, retransplantation makes a significant contribution to overall long-term survival in children undergoing LT [82].

Nonsurgical Complications

Infections

Infection is a common complication of LT. The majority of transplant recipients will have at least one episode of infection in the postoperative recovery period, particularly if transplanted for acute liver failure, if there is persisting graft dysfunction or following steroid therapy for acute

rejection. Bacterial pneumonia is the most common infection in the first week. Gram-positive line infections occur from 5 days onwards and venous access must be changed regularly. Gram-negative sepsis is often associated with biliary leak, bowel perforation or graft ischaemia. Immunosuppressive therapy may minimize clinical signs of sepsis. Late opportunistic bacterial infections including legionellosis, nocardiosis and tuberculosis may also occur particularly in those children who have received higher levels of immunosuppression. Of 2291 children (<18 years) reported by Shepherd et al., infection was the primary cause of death in 3.1% of all recipients, primarily due to bacterial sepsis (56%), with viral and fungal infection accounting for 19% and 10%, respectively, of infection deaths [83]. Infection contributed directly or indirectly to 46% of all deaths from multi-organ and cardiopulmonary failure.

Viral infections tend to occur later than bacterial, and the overall incidence of CMV disease was 6%, EBV disease 8.6% and lymphoproliferative disease (PTLD) 2.7%. Multivariate analysis showed that risk factors for bacterial infection included age (infants vs adolescents), race (black and Hispanic vs white), IS (cyclosporin vs tacrolimus), year of LT (before 2001 vs after 2001), serum bilirubin level and organ donor type (deceased donor split or reduced sized). Risk factors for viral infection were treated acute rejection, era of transplant (before 2001) and organ donor variants (split or reduced sized, i.e. older donors). The risk from infection exceeds that of rejection, particularly in infants, who have three times the rate of bacterial or fungal infection of that seen in adolescents. Infection was the most common cause of death with a tenfold greater risk of death from infection than from rejection (3.1% vs 0.2% of patients; 46% vs 4.7% of deaths, $p < 0.001$).

Fungal infections particularly *Candida* respond to treatment with fluconazole or amphotericin B, but invasive aspergillosis may be fatal. Fungal sepsis is particularly common in children with acute liver failure or bowel perforation, and they must receive appropriate prophylaxis. Atypical pneumonia due to *Pneumocystis carinii* should be considered if hypoxia is present and treated with high-dose septrin. Central nervous system infection with toxoplasmosis is rare and should be treated with pyrimethamine/sulfadiazine or high-dose penicillin.

Herpes viruses form the most important viral pathogens post-transplant. Herpes simplex and Varicella Zoster infections occur within 1 and 3 months, respectively, post-transplant, and both respond to early treatment with acyclovir. Cytomegalovirus infection occurs between 2 and 6 weeks post-transplant and should be treated by a reduction of immunosuppression and ganciclovir. High-risk cases, for example, CMV-positive donor and recipient, are routinely given prophylactic ganciclovir. CMV hepatitis presents with flulike symptoms, high fever and relative neutropenia with a mild rise in serum transaminases. Serology is of limited value and has been replaced by diagnostic tests which monitor levels of antigenaemia or CMV DNA.

Lymphoproliferative Disease and Epstein-Barr Infection

Epstein-Barr virus (EBV) infection may occur as a primary infection from a positive donor or reactivation of a previous infection [84]. It presents with a spectrum ranging from a mononucleosis-like illness to a malignant lymphoma. The presence of unexplained persistent fever, anaemia and positive faecal occult bloods are suggestive of PTLD and should prompt endoscopy and CT examinations. Young, previously EBV-negative children appear to be particularly at risk of developing EBV-related PTLD. The overall incidence is 2% but may be as high as 5–10% in children less than 1 year. It is a life-threatening complication with 35% mortality [85, 86]. Risk factors include primary EBV infection, age at LT, type and intensity of IS and CMV infection [87]. Because most children have not been exposed to EBV, they are particularly susceptible to acute infection, and in combination with IS they are at high risk of developing PTLD. Infants have 10 times the rate of PTLD of adolescents presumably because they are EBV and CMV naïve and have an immature immune system. Some PTLD will regress with immunosuppression reduction and steroid therapy; however, rituximab has become the mainstay of therapy with systemic chemotherapy being reserved for children not responding to this treatment.

An individualized approach to the prevention of EBV disease and PTLD is helpful in infants, given their risk. EBV PCR monitoring identifies children with increasing viral load and allows for IS reduction. The therapeutic value of preventive use of antiviral therapies (e.g. ganciclovir or acyclovir) remains unproven. However, improved EBV monitoring has led to earlier diagnosis of PTLD with less systemic involvement resulting in more favourable outcomes.

Box 76.3 Medical and surgical complications (incidence)

- Post-transplant lymph proliferative disease (1–15%)
  - Renal dysfunction (1–32%)
  - Hypertension (4–50%)
  - Post-transplant metabolic syndrome (18–67%)
  - Hyperlipidaemias (7–26%)
  - Neurocognitive function (15–30%)
  - Disease recurrence: BSEP, PSC (0–33%)
  - Food allergies (5–38%)

Graft and Patient Survival

Over the past 5 decades, there has progressive improvement in outcome after LT. According to data from the UNOS, adjusted graft survival for both deceased and LDLT programmes have achieved excellent survival rates in children. Patient survival at 1 year after elective LT should be in excess of 95% and for ALF approximately 80% and of the order of 80% and 70% at 10 years, respectively. Graft survival has been improving with lower rates of retransplantation due fewer technical complications and a lower incidence of chronic rejection. Concerns over the finding of significant graft fibrosis in many children at 10 years post-LT have raised concerns over the longer-term outlook and question whether IS levels are appropriate [88]. It has been shown that increasing IS in the presence of fibrosis does lead to improvement in the liver fibrosis. Long-term outcomes are similar for all children except for the very young <3 months and young adults. The reasons for the lower outcome of adolescents are not clear and require further research.

Quality of Life

The improved long-term outcomes are turning attention to quality of life of survivors. Quality of life is difficult to measure, especially in children. Studies show that most children surviving more than 1 year post-LT were able to return to school (31). Quality of life is dependent on good graft function and an absence of recurring medical complications requiring repeated hospital admission.

Overall quality of life seems to be satisfactory in children with good graft function. Growth, social and physical developments are normal in the absence of liver diseases such as Alagille. Alonso et al. on behalf of the Studies of Pediatric Liver Transplantation (SPLIT) group studied 873 children surviving at least 12 months, mean age of 8.17 ± 4.43 years (from 22 centres) [89]. Using a 23-item PedsQL 4.0 (Mapi Research Institute, Lyon, France) Generic Core Scales, which encompasses physical, emotional, social and school functioning, they demonstrated that these children had moderately diminished HRQOL as compared with healthy peers. The largest difference was noted in the area of school functioning, with days missed from school being important from the perspective of children and their parents. Comparisons in HRQOL in this study with children with cancer revealed that both populations shared similar struggles in social and school functioning areas.

Some effect has been noted on cognitive function; however, motor developmental delay may be due to chronic illness. Parents often comment that their child is active and plays well and is able to do physical activities. Overall family interaction and school behaviour also improve. Parents may view themselves as more relaxed and being able to be more balance and consistent in applying discipline. Siblings behave more appropriately but may remain resentful towards the recipient. For many families, there is difficulty in adjusting to the new situation of having a well rather than ill child. There remain long-term anxieties about the child's prognosis and well-being. The child is used to taking a disproportionate amount of the parents' time and attention at the expense of other siblings and trying to restore "fairness" can cause problems. Behavioural immaturity may persist, and acts of defiance or aggression are sometimes seen particularly in teenage years. There is continuing parental concern over rejection, side effects of IS, of being overprotective, continuing medical and "social" expenses and changes to family dynamics. Parents who have devoted their energies into the care of a chronically ill child sometimes interrupting their professional life may find it difficult to adjust to their new role within the family [90]. Anxieties about children going to school and being separated from their parents after many years of dependence can also be stressful [91]. However, these challenges can be addressed with continuing follow-up and support from healthcare professionals within the transplant network.

A recent study examined the change in health-related quality of life (HRQOL) and cognitive functioning from early childhood to adolescence in liver transplantation recipients. In 8 North American centres through the Studies of Pediatric Liver Transplantation consortium, a total of 79 participants, aged 11–18 years, previously tested at age 5–6 years in the Functional Outcomes Group study were identified as surviving most recent LT by 2 years and in stable medical follow-up. According to parents, adolescents had worse HRQOL and cognitive functioning compared with healthy children in all domains. Adolescents reported HRQOL similar to healthy children in all domains except psychosocial, school and cognitive functioning ($P = 0.02$; $P < 0.001$; $P = 0.04$). Participants showed no improvement in HRQOL or cognitive functioning over time. For cognitive and school functioning, 60.0% and 50.8% of parents reported "poor" functioning, respectively (>1 standard deviation below the healthy mean). Deficits in HRQOL seemed to persist in adolescence with one half of adolescent LT recipients at risk of poor school and cognitive functioning, likely reflecting attention and executive function deficits [92].

Transition to Adolescence and Young Adult Life-Adulthood

The most difficult step in long-term follow-up is the transition from childhood, and LT recipients require specific care, attention and support. Life cannot be considered to be normal because of the need to take IS and lifestyle restrictions regarding alcohol. The appearance of conflict between recipient and their parents may become problematic. Maintaining a healthy relationship will improve adherence and the quality of life of the recipient. Adolescent and young adults are concerned about their appearance, and

complications such as acne, hirsutism and gum hypertrophy may lead to non-adherence unless IS is changed to help. Nephrotoxicity is a significant problem, and by 10 years post-LT, up to 5% of recipients may have developed end-stage renal failure which has a major bearing on long-term follow-up [93].

An increased incidence of graft loss in adolescence has consistently been linked to non-adherence and has become a leading cause of late death in this population. Annunziato at el [94]. examined adherence during transition and compared it with paediatric and adult cohorts. Adherence was significantly poorer in the adolescent cohort vs paediatric and adult cohorts. The increase in non-adherence was due to a number of factors: paediatric clinics have a more "hands-on" approach to treatment; changes in insurance status may be associated with a lapse attendance and IS usage; and the stress of transitioning to less familiar providers may exacerbate existing individual and familial risk factors that compromise adherence. Subsequently, the same group tested a pilot intervention of facilitating transition [95]. Twenty-two patients were enrolled in a two-session educational programme providing details of their underlying liver disease and treatment. A second component focused on educating families regarding the transition of healthcare responsibility to the recipient facilitated by a clinical psychologist. This structured intervention significantly improved adherence with improvement in liver function and greater consistency of tacrolimus trough levels. Fredericks et al. also confirmed in a review that rates of non-adherence among adolescents are high, and adherence to IS is a critical factor in transition with an increased risk of poor long-term health outcomes [96]. Further research is needed to identify factors and interventions that affect long-term health outcomes in this population.

Long-Term Outlooks and Trends

Long-term survival is now achieved by large numbers of children following LT. The initial obstacles to survival, such as getting to transplant and managing immunosuppression, are being tackled, but the challenges raised by psychological, social and mental health problems produced by successful transplantation are belatedly being addressed by specialist transition and young adult services. The overall life expectancy is still to be determined for a child undergoing liver transplantation. The goal remains transplantation without long-term immunosuppression, but until donor-specific tolerance can be safely induced, there will continue to be complications from IS therapy. As the majority of children are under 5 years of age at the time of transplant, continuing specialist care and education must be provided, particularly during transition to young adult life, when life's challenges may threaten well-being and non-adherence to medication. Ensuring that they complete their education, have employment and are able to have families of their own is important and in the future may have a bearing on the timing of transplantation. The challenge is to ensure children undergoing liver transplantation can be restored to a normal life expectancy with the potential to enjoy life to the full.

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Growing Up with Liver Disease

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Marianne Samyn, Jemma Day, and Anna Hames

Introduction

As the survival of children with liver disease has significantly changed over the last few decades, pediatric liver transplantation and improved medical and nutritional management have changed the outcome for children with liver disease and a large cohort of patients is now moving into adolescence and adulthood with a liver condition. Unfortunately, young people with liver disease have inferior health outcomes compared to younger and older age groups [1]. They face challenges inherent to their adolescent development, now known to carry on into the mid-20s, but also in particular adult healthcare professionals might not be familiar with childhood liver diseases and their management [2, 3]. The current setup of healthcare systems sees young people looked after by either pediatric or adult services, irrespective of whether this is developmentally appropriate and the current provision of support during the transition from one service to the other is limited [4].

In this chapter we, as physician and clinical psychologist, will give an overview of the interaction of physical development during puberty and liver disease, as well as the psychosocial and health behavior aspects of adolescence. We will share our experience of running an integrated multidisciplinary care model for young people with liver disease aged 16–25 years.

Young People

The World Health Organization recognizes that "young people" aged between 10 and 24 years are a population who require dedicated care [5]. Having a chronic condition or disability has multiple effects on adolescent development including biological, psychosocial, and social effects that can in turn contribute to poor adherence and risk-taking behaviors [6]. Non-adherence to medication is a particular challenge in the adolescent population as it is difficult to measure, often multifactorial however relatively developmentally appropriate. Its prevalence is reported to exceed 50% in the post-transplant population and effects long-term outcome in this patient population [7]. Adolescence coincides with transfer of medical care from pediatric to adult-centered services hence the importance of defining a dedicated, individualized transition care pathway for young people. This will be discussed in more detail further in the chapter.

Medical Aspects of Growing Up with Liver Disease

Within pediatrics, liver disease is a relatively new specialty within which the last few decades have seen a significant change in the diagnosis and management of conditions. Patients tend to present in infancy or later childhood with a variety of genetic and incidental conditions, either in an acute, often life threatening, or more chronic setting. Lifelong specialist follow-up and treatment are usually required. The development of pediatric liver transplantation has had a significant impact on the outcome and prognosis of children developing end-stage liver disease or presenting with acute liver failure, and the majority of the patients are now moving into adolescence and adulthood. This emerging population is a challenge for both pediatric and adult hepatology teams.

Information on the long-term outcome of patients with liver disease presenting in infancy, such as biliary atresia (BA) and Alagille syndrome, is becoming available but is still scarce and more focused on survival data. It is estimated that 14–44% of patients with biliary atresia survive into adulthood without needing liver transplantation. In our experience 28%

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of BA patients survived with their native liver up to 16 years with a quarter requiring liver transplantation during adulthood. Abnormal serum bilirubin levels, evidence of portal hypertension with varices on endoscopy, and having an episode of cholangitis during adolescence were associated with the need for liver transplantation in adulthood [8]. Of note is that listing criteria vary significantly between pediatrics and adults, and the use of mathematical models based on adult liver diseases disadvantages young people. We found that when comparing a group of young people with BA (median age 15.5 (range 13.8–18.6) years) either listed by the pediatric ($n = 22$) or adult team ($n = 14$), those listed by the adult team waited significantly longer on the waitlist and more likely to require intensive care support at time of listing (29% vs 5%; $P < 0.05$), and this was independently associated with poorer patient and graft survival. The mathematical models used by adult teams as listing criteria did not correlate with waiting times or outcomes. What did improve survival was the support from the multidisciplinary young people's liver service with all young people in this group ($n = 11$) surviving compared to 88% in the rest of the group [9].

In Alagille syndrome, extrahepatic aspects of the syndrome related to vascular or renal involvement are becoming more relevant and can impact the long-term prognosis [10, 11].

The advances in molecular genetics are now enabling us to diagnose genetic liver conditions such as familial intrahepatic cholestasis and other rarer metabolic conditions such as mitochondrial cytopathies, etc. The implications of dealing with a genetically based condition can have further long-term implications on adult life and prognosis [4].

Other conditions such as autoimmune liver disease, Wilson disease, and nonalcoholic fatty liver disease tend to present more frequently during adolescence, and patients will have to come to terms with their condition and management during an already challenging time in their life.

Finally, some young people will present to adult services with liver-related complications related to conditions such as complex congenital heart disease or childhood cancers and will require specialized care. In a series of 95 patients who had underwent a Fontan operation during infancy, 23% developed Fontan-associated liver disease which has shown to be linked with morbidity and premature mortality [12]. Regular screening for adolescents and adults has now been recommended.

Outcome Data

Whereas long-term outcome following pediatric liver transplantation is significantly better compared to adult cohorts (21–52%) with up to 20-year patient and graft survival of 79% and 64%, respectively, those transplanted between the ages of 12 and 17 years have inferior patient and graft survival, and this is similar for other solid organ transplants as heart, kidney, and lung transplants [13, 14]. Young adults, aged 18–24 years, experience disparities both while waiting for transplantation and with regard to outcome [15].

Young people (12–25 years) hence constitute a unique and vulnerable cohort who deserves special attention by health professionals in order to improve survival.

In the non-transplant setting, a recent report on predictors of poor outcome in a cohort of 133 patients with autoimmune hepatitis aged 14 and over found that presentation between the ages of 14 and 20 years was a significant independent predictor of liver-related death or requirement for liver transplantation, suggesting that their condition was more challenging to manage compared to the older population [16].

Impact of Liver Disease on Physical Development

Growth retardation is common in children with chronic liver conditions and more common in cholestatic liver disease, where some degree of catch-up growth is noted after liver transplantation. In an analysis of growth following liver transplantation, risk factors for poor linear growth were prolonged steroid exposure, lower weight percentiles at time of transplantation, linear growth impairment pre-transplantation, and metabolic disease as primary diagnosis [17]. More recently, out of a total of 892 liver transplant patients between 8 and 18 years, 20% had linear growth impairment at their last follow-up and, where available, height z-scores were significantly lower than the calculated mid-parental height z-scores. Linear growth impairment at transplant, re-transplantation, non-white race, and primary diagnosis other than biliary atresia were found to be independent predictors of growth impairment. In the same study, the authors reported that on the pubertal development of 353 children, 61% of girls and 58% of boys aged 16–18 years reached Tanner 5 compared to 100% of a normative population with growth impairment occurring in 11% of Tanner 5 subjects [18]. Growth impairment has also been described in genetic conditions such as Alagille syndrome and can also be associated with the treatment. Further data is needed to establish the prevalence of growth failure and pubertal delay in chronic liver disease but is available in other chronic conditions such as inflammatory bowel disease, nephrotic syndrome, asthma, and cystic fibrosis. Growth failure and pubertal delay can have a significant psychosocial effect on quality of life and long-term outcomes; hence treatment of recombinant human growth hormone in this population has been reported to be associated with improvement in psychosocial functioning as well as linear growth [19]. Larger studies are needed to assess its safety in this patient population.

In girls with chronic liver disease, menstrual cycles can be irregular, and amenorrhea and anovulation are common. Menorrhagia can occur in patients with advanced liver disease with portal hypertension. Estrogens, and typically the synthetically produced ethinylestradiol used in the combined hormonal preparations, are more potent and have a potential effect on the liver irrespective of the route of administration. Progestogens do not have receptors on the liver cells and are commonly given at a lower dose and well tolerated.

Although not contraindicated in patients with compensated cirrhosis, both in pre- and post-liver transplant setting, current contraceptive recommendation is with progesterone-only preparations such as minipill (e.g., Cerazette), medroxyprogesterone injection or etonogestrel implants, and, if sexually active, levonorgestrel releasing intra-uterine system [20]. Successful pregnancy outcomes have been reported both in chronic liver disease and liver transplantation settings, although there is an increased risk both for mother and baby. Treatment with calcineurin inhibitors, steroids, and azathioprine is recommended to be continued during pregnancy to avoid graft dysfunction or relapse in autoimmune liver disease; however, mycophenolic acid and rapamycin are contraindicated because of the increased risk of birth defects. In patients with portal hypertension, an upper GI endoscopy during the second half of the second trimester is indicated to assess the degree of portal hypertension and the need for further management to avoid GI bleeding during the course of pregnancy [21]. Obstetric follow-up by an experienced team in a hospital setting is required. Adolescent girls should be informed timely of the various contraceptive options, the potential complications of pregnancy and childbirth, as well as the possible genetic implications of their underlying condition.

Cosmetic side effects of medical treatment such as steroids and currently less commonly use, cyclosporine, can have an impact on body image and adherence to treatment in the adolescent population (see later), and health professionals should keep this in mind when prescribing treatment.

In order to effectively manage young people's care, it is crucial to successfully address their wider "medical, psychosocial and educational/vocational needs" [22]. In order to do this, professionals need to be familiar with the unique developmental stage of adolescence and recognize that young people are neither just "big children" nor "small adults." This developmental perceptive is discussed in the next section, along with the psychosocial elements of growing up with liver disease.

Adolescent Development and Its Interaction with Liver Disease

The biopsychosocial changes associated with adolescence interact with how young people manage their illness and treatments and accordingly with how their healthcare should be approached. For an excellent review of this area, please see Suris, Michaud, and Viner [6].

Although most of the literature focuses on adolescence, research demonstrates that structural and functional changes continue to take place in the brain into young adulthood [23]. This is mirrored by changing societal norms with young people increasingly delaying many of the traditional "tasks" of adulthood, such as financial independence and starting a family. Furthermore, health outcomes are poorer for young adults into their mid-twenties, so it is more helpful to think about young adult development more broadly [24].

Adolescence is traditionally defined by the onset of puberty. Delayed puberty, reported in young people with liver disease, can impact on how the young person views themselves, their illness, and their wider world. For example, an adolescent who looks younger may be treated differently by people and have reduced social opportunities. Common stories from patients include being asked by adult clinic staff whether they are there with their mum, being stopped by the police when driving to check their age, and being refused entry to 18-rated films or pubs.

Alongside the physical changes are changes in how young people think and feel and in the nature and importance of their social world. In order to become an independent adult, the adolescent needs to separate from their parents. They start to develop a more independent sense of identity, and their peer group typically takes over from family as being their main social world [25]. Peer acceptance becomes key, with a strong desire to feel normal. Self-consciousness increases. The typical adolescent has an increased sense of invincibility, poorer abstract thinking, and reduced thoughts of the future [26]. An increasing body of research demonstrates ways in which the structure and function of the adolescent's social brain is distinctly different from that of children or adults [27].

As part of this adolescent profile, risk-taking behaviors peak, with high levels of alcohol and drug use, smoking, and unsafe sex [28]. In the UK, "binge-drinking" is widespread and synonymous with certain rites of passage for young people, such as the introductory "freshers' week" at university. Young people growing up with liver disease have the same needs as other young people, with the additional challenge of trying to balance their health needs against their social and psychological needs. The way to meet these can often seem to be opposition. Indeed, research suggests that alcohol and drug use is similarly prevalent in young people with chronic illnesses as compared to their healthy peers [29] and they are equally as likely to be sexually active [30]. This adolescent profile and tendency to take risks helps young people to develop independence but can present significant challenges for successfully managing a chronic illness. This is discussed further below under adherence.

Impact of Family

All of the above changes happen within a family context. To enable the young person to develop into an adult, their family also need to adapt their roles, for example, by giving their child more freedom and privacy [31]. Parents/carers of children with chronic illness have often dedicated much of their lives to caring for their sick child and have their own relationships with the illness and hospital teams. Young people's relationships with their family may be impacted by their liver disease, for example, having less independence. Parents/carers might also have had to change other roles in their lives, such as giving up work, in order to successfully care for them. This can result in some parents seeming to be more overprotective, for example, worrying about their adolescent taking their medication, abstaining from alcohol at parties, monitoring for symptoms, or appropriately seeking help [32].

There are also significant challenges for families of young people who are diagnosed with a liver disease during their adolescent years, as is common, for example, in autoimmune liver disease or Wilson disease. At a time when adolescents should be becoming more independent, the acute stage of illness forces them into a state of dependence on others. This can present challenges for the whole family that may not be expected at this stage of development [33], such as parents needing to take time off work, physically caring for their child and spending concentrated time together that might not otherwise have been expected from being the parent of a teenager or be normal among their peers.

Wider Influences

The above processes occur within a wider set of systems still, such as schools, workplaces, friendships, and other relationships; how the liver disease is managed in any of these contexts will interact with the young person's adjustment and management of it. Furthermore, this is within a societal context in which the general public hold certain beliefs, assumptions, and prejudices. As public perception of people with liver disease and transplant is most commonly associated with drug and alcohol use, young people struggle to develop a positive self-identity if they associate (or other people associate them) with this stigmatized group. Our patients often grapple with the dilemmas about who to tell about their condition and how to tell, as many have experienced bullying or prejudiced comments in the past. It can be beneficial to discuss these dilemmas with patients and help support them in communicating their needs to schools and workplaces.

Given the importance of peer relationships during this period, it is worrying that peer networks are often disrupted in young people with chronic illness [34]. Among young people, post-transplant peer support has been found to be an effective means of engaging young people in services and improving their health outcomes and well-being [35]. In a small recent study in which young people were trained to act as mentors for younger post-transplant patients, the mentors themselves benefited from improved adherence as well as the mentees (measured by lowed mean tacrolimus standard deviation levels) [36]. The authors suggested that this may be attributable to the increased emotional support from attending the mentor training workshop. As part of the liver transition service at King's College Hospital, we run peer support days and peer mentoring for young people with chronic liver disease and post-transplant. Preliminary feedback suggests numerous benefits of this for both young people and their mentors, including feeling more positive about having a liver condition due to increased hope and feeling less alone, feeling more prepared for transition, and several comments akin to "I wish I had something like this when I was younger" [37].

Most of the developmental models are based upon Western notions of adolescence. It is unclear how this may differ in other cultures, for example, where adolescence may not exist as a construct or notions such as independence from family are not expected or endorsed. How culture interacts with chronic illness management and transition is an under-researched area that demands further attention [38]. It is important for professionals to be curious about what the adolescence and their family expect at this stage of development rather than making assumptions about how these constructs may or may not apply.

Psychological Aspects of Growing Up with Liver Disease

Adolescence is a period of rapid change, full of opportunities and challenges. Young people growing up with chronic liver disease have the same aspirations in life as their healthy peers but have additional stresses and restrictions to manage, including hospital visits, time off school, medication, and lifestyle restrictions. Rather than focusing purely on the presence of psychological distress, it is important to consider how all young people and families adapt to their changing health needs at different stages of development. Most young people, with or without liver disease, strive to be normal [39]. Those who have difficulties adjusting to their illness/treatment and integrating it into a positive self-identity are at increased risk of developing psychological difficulties and are less likely to manage their physical health needs effectively. Routine questions about the adolescent's wider world are crucial for engagement and in order to assess how they

are adjusting to the demands of their condition and areas that may require intervention [40].

There has been relatively little research into the psychological needs of young people growing up with liver disease, and most of this limited research has focused on those post-transplant. Research conducted with adults with liver disease is unlikely to be generalizable as the populations are different on multiple levels, including age and developmental stage, age at diagnosis, type of liver disease, and/or reason for transplant. A brief overview of some key areas is given below, citing research specific to liver disease where it exists and otherwise extrapolating from other chronic childhood diseases.

Quality of Life

Quality of life is a broad term that encompasses a range of physical, psychological, and social factors. Most studies investigating quality of life in young people with CLD report only on those post-liver transplant, rather than adolescents with CLD as a group. Health-related quality of life is found to be poorer in children and young people post-liver transplant as compared with the general population, but similar to young people with other chronic conditions, including other solid organ transplants [41, 42]. Across studies, Ohnemus identified predictors of poor QoL included transplantation in adolescence (as well as sleep problems and medication adherence) [43]. A study of children and adolescents with autoimmune liver disease found a similar trend, with poorer quality of life being associated with the presence of symptoms such as ascites, abdominal pain, and fatigue [44].

In a qualitative study aiming to understand how liver transplant affects young people's quality of life, adolescent participants spoke about the impact of transplant on their relationships, schooling, fatigue, burden of medication, communication with healthcare professionals, and thinking about the future [41]. These are key areas to explore when working with young people and demonstrate the importance of fostering good collaborative relationships with young people, in which they feel listened to and valued and their wider needs and hopes are respected.

School Achievement

A young person growing up with a chronic liver disease or transplant is more likely than healthy peers to take time off school for hospital appointments and ill health. This can have a significant impact on their school attainment and subsequent employment opportunities in adulthood. There is also some evidence that a portion of the poorer QoL documented in this population relates to poorer cognition and school performance. For example, Ohnemus and colleagues [43] found adolescent liver transplant recipients reported QoL similar to healthy peers in all domains except psychosocial, school, and cognitive functioning. Furthermore, these results indicated no reported improvement in cognitive functioning over time, suggesting transplant does not "fix" this problem.

Data available on cognitive development in the context of pediatric liver conditions, and mainly in the post-liver transplantation setting, confirms an increased incidence of learning disability in this population. Out of 144 patients from the SPLIT registry children aged between 5 and 7 years and more than 2 years post-transplant, 26% were found to have a mild to moderate, and 4% a serious, learning disability with 25% having impaired performance with reading and math skills and a relevant executive functioning deficit which would potentially affect independent management of their health condition in adult life [45]. Further research identified height centile at transplantation and genetic-metabolic conditions as having a high impact on long-term cognitive functioning [46].

The literature relating to cognition in our young people is in its infancy. The limited evidence so far does indicate that cognition is poorer in children and young people with CLD [47] but there is insufficient data to determine whether cognitive development differs between young people surviving with their native livers and young people undergoing liver transplantation. Studies tend to focus on early childhood rather than adolescence or young adulthood and disproportionately on those already post-transplant and not those surviving with their native liver [48–50]. Studies are also heterogeneous due to sample size, age, condition, areas examined, and tests used.

A recent systematic review of the available literature identified a total of 25 studies which have investigated cognition in children and young people with liver conditions (n = 1913) [47]. The majority of these (19/25 studies examined) described individuals post-transplant (n = 1372 children). Of those surviving with their native livers, four out of six studies found low average or impaired scores on cognitive and behavioral measures [51–53]. These studies did collectively indicate that the poorer cognition observed persisted into adolescence, with approximately 50% of young people scoring below 85 for IQ tests (compared to expected rates of around 15% in the general population) [54]. There is also evidence of poorer educational attainment, which is likely to be related to lower cognition, and at levels over and above those with comparable school absence due to hospitalization for other forms of chronic illness [55]. With poor quality of life and job performance seen into adulthood, the importance of interventions to target these impairments becomes increasingly clear.

The longer-term impact of these childhood difficulties also needs to be further researched, as in clinical practice we frequently see the long-term consequences such as in Case Study 77.1.

Case Study 77.1 Harry was diagnosed with biliary atresia shortly after he was born. Following a Kasai procedure, he required regular visits to hospital for appointments and sometimes needed admission for treatment with antibiotics or endoscopy procedures as he developed portal hypertension. He was a bright, sociable child who was well liked by patients and staff. Aged 11, his health deteriorated and the decision was made to list him for liver transplantation. A year later Harry was transplanted and he recovered well after surgery. Due to time spent in hospital, he missed most of his formal education and left school without any qualifications. Harry is now 22 years old. He lives with his parents and is unemployed. He has held a number of causal jobs, but struggles to find permanent employment due to his lack of qualifications and relevant experience. Harry feels left behind by his friends, most of whom who have now been to university and started good jobs.

Mood Difficulties

Research investigating psychological well-being in adolescents with chronic illness more broadly suggest that there are higher rates of depressive and anxiety symptoms relative to healthy controls, but the rates vary across studies and illness group (see meta-analysis by Pinquart & Shen [56]) and were particularly common in young people with conditions impacting upon energy levels, those with severe symptoms, and those resulting in a visible difference, all of which can apply to young people with CLD. Symptoms of chronic illness, restrictions on functioning, and the need for complicated treatment regimens are likely to interfere with many aspects of adolescent life and to cause frustration.

Post-traumatic Stress Disorder

Young people with chronic liver disease are likely to have had some unpleasant experiences in hospital and times which may have been felt confusing, scary, or upsetting. High rates of post-traumatic stress disorder (PTSD) have been found in adolescents who have had transplants. For example, in 104 adolescents (aged 12–20 years) post solid organ transplant, 16% met full criteria for PTSD, with an additional 14% reporting 2 of the 3 necessary symptom clusters at a level that was causing them clinically significant distress [57, 58] reported similar prevalence rates of PTSD (13%) in 76 children post-transplant; these PTSD symptoms were significantly under-reported by parents.

In addition to the distress associated with PTSD, in a small study of 19 adolescents post-liver transplant [59], a significant association was found between presence of PTSD symptoms and non-adherence (as measured by blood levels and clinician judgment). This is likely to be due to medication serving as a reminder of the transplant and non-adherence therefore being a form of avoidance. Functional outcomes are also found to be lower, for example, adult survivors of childhood cancer who had PTSD were found to have lower functioning in areas such as school, work, and personal relationship [60].

Failure to identify PTSD compromises the young person's well-being, impacts on their functioning as adults, and is associated with non-adherence. As parents tend to underestimate rates of PTSD and there is no relationship between the objective characteristics of the trauma and the risk of PTSD [61], it is impossible to predict who will have difficulties. Detection therefore relies upon directly asking the young person. Research has not addressed rates of PTSD in young people with chronic liver disease more generally, but as these young people also encounter situations where they perceive their life to be threatened, then it is reasonable to assume that their rates of PTSD may also be elevated.

One of the most significant challenges of caring for adolescents is the high rates of non-adherence. This is outlined below.

Adherence

As highlighted earlier, rates of non-adherence to medication are found to be as high as 50% in adolescents post-transplant, with significant negative implications for their health. Although non-adherence to treatment, medical advice, and clinic appointments is considered developmentally appropriate in this population, it is a concern for clinicians working with young people and is often challenging to manage.

It is easy to see that a typically developing adolescent as described in the earlier section might not take all their medications or attend appointments correctly. Increases in impulsivity, delay discounting (the extent to which consequences decrease in effectiveness to control behavior as a function of there being a delay to their occurrence), reward-seeking, and emotional reactivity are noted in adolescence, which make this period a time of heightened vulnerability to taking risks with their health. Adherence in liver disease or transplant requires the patient to trust their doctor that the treatment is

required, buy into the notion that the status of their liver disease may not correspond with symptoms, be motivated by a long-term outcome of improved health, and be able to plan and organize themselves to maintain a good routine. This directly contrasts with the developmental profile of young people, as illustrated in Case Study 77.2.

A full review of the factors associated with non-adherence is outside the scope of this chapter, but a number of comprehensive reviews exist (e.g., Drotar, 2009; Shemesh et al., 2008, Kyngas, Kroll & Duffy 2000) [62–64]. Many of the characteristics known to make adherence more difficult are present for young people with liver disease; for example, the treatment is seemingly preventative rather than curative, does not have any immediate tangible benefits, and needs to be taken for life. Knowledge is generally necessary, but not sufficient for adherence (e.g., Macquaid, Kopel, Klein & Fritz, 2003 [65]), and requires particular attention as young people grow up and each with different understandings.

Case Study 77.2 Jake is 18 years old. He was well throughout his childhood until being diagnosed with autoimmune liver disease when he was 14 years old. When he was first diagnosed, Jake felt quite unwell and spent a week in hospital, but since then he has been well and only has to go to an outpatient clinic appointment every few months. Jake does not think of himself as being sick and doesn't really think about it except for when he goes to hospital. He is most bothered about the way he looks, in particular about his acne, which started when he was commenced on steroid treatment and lack of muscle tone. He feels very self-conscious around other people his age and often feels quite down about his appearance. Jake is told to take daily medications to prevent him from getting ill again in the future - but when he stops taking his medications, nothing bad happens; he actually feels better because he isn't bothered by side-effects, his skin gets better and his face looks more defined. Jake feels happier because he feels more similar to his friends, and doesn't feel so self-conscious about having to remember to take his meds or risk having to explain his condition to others. Jake's been told he shouldn't drink alcohol, but all of his friends do and it's really hard to explain why he can't. When he started drinking recently at a party it was really fun and nothing bad happened, so he thinks it must be alright. Jake doesn't really get on very well with his parents and teachers at the moment, so doesn't tell them because he knows they will nag him about it. When he goes to hospital his mum does most of the talking. His doctor tells her about some blood tests numbers that he doesn't really understand, and then he gets to go home.

Exploring non-adherence should be part of the routine management of all patients (irrespective of age) and approached in a non-judgmental fashion to encourage disclosure and engagement. Health professionals should know that in young people non-adherence is considered to be relatively developmentally appropriate and not suggestive of distrust in healthcare professionals or equally rejection on the part of the adolescent. Individual education, which is tailored to the young person's needs and repeated and checked regularly, is important, to ensure the young person has a good understanding of their condition and rationale for treatment recommendations. It is important to understand the young person's priorities and encourage them to have open conversations about the barriers to adherence for them; for example, discussions may enable medication regimes to be simplified or altered to fit in with the person's routine, encouraging use of alarms and reminders, taking medication on sleepovers, and more broadly how to manage the handing over of responsibility from parents to their child. Simplifying medical treatment and conversion to once daily preparations of immunosuppression have been reported to improve adherence and treatment satisfaction [66]. An overview of strategies for improving adherence can be found in Table 77.1.

Non-adherence has also been found to be associated with psychosocial distress, such as PTSD [59] and other psychosocial stressors [67]. Given poor mental health is linked to worse physical health via increased non-adherence to medication and disengagement from services, it is important that mood and emotional well-being in young people post-liver transplant is considered routinely, as part of good clinical care [68, 69]. Social difficulties such as financial restrictions should also not be overlooked. For example, in the UK at the age of 18 years, young people have to start pay-

Table 77.1 Adherence management strategies

Barrier to adherence	Strategies
Naive about the risks of non-adherence	Individualized education about illness and medication
Burden of medication regime: too many tablets or too many times a day	Simplify medication regimes Ensure young person understands rationale for each medication and anticipated course
Non-intentional non-adherence: forgetting or organizational difficulties	Pill boxes, blister packs Medication charts and apps Alarm reminders Visual reminders
Intentional adherence: choosing not to take it due to the meaning of the medication	Explore beliefs about illness and medication, including the benefits of non-adherence for the young person. Assess mood Referral to psychologist
Intentional adherence: practical barriers	Assess barriers such as housing, finances, parental support Referral to social worker

Table 77.2 Routine assessment and management of adherence

Task	Rationale	Example questions
Engage the young person	Young people are more likely to be actively involved in their healthcare and more adherent if they have a good relationship with their healthcare provider Screen for psychosocial difficulties Gather information about how the illness and treatment fit into the young person's life	See HEADSS (Goldenring and Cohen [40]): ask about home, school/college, friendships, activities, and interests
Assess who is responsible for medication	Responsibility needs to be handed over from parents to young person: difficulties often arise during this transition	"Who is in charge of medication at home? How long have you been taking charge of your medication? Who organizes the prescriptions?"
Assume non-adherence and routinely assess with every patient	Rates of non-adherence exceed 50% – most young people will be non-adherent some of the time Impossible to predict who will be non-adherent so need to ask everyone Asking questions in a non-judgmental way that assumed some non-adherence is more likely to increase disclosure	"In a normal week, how often do you tend to miss your medication? How often do you take it at a different time?"
Normalize: full adherence is difficult; very few people are adherent all of the time	More likely to increase honest disclosure and willingness to discuss the barriers to their adherence Trying to scare or tell off your patient is unlikely to improve their adherence but will ensure that they don't disclose it to you again!	"Most young people we see struggle to take all of their medication all of the time. We know that it can be a really hard thing to have to take medication every day."
Check understanding of illness and risks of non-adherence	Knowledge is necessary (but not sufficient) for adherence Need to ensure that the young person understands why they need to take medication and fully understand the risks of not taking it	"How would you explain your condition to someone who hadn't heard of it before? What do you think the medications do? What do you think would happen if you didn't take your medication? How many doses of medication do you think you could get away without taking?"
Assess intentionality of non-adherence	Different determinants of non-adherence require different interventions	"Are there times that you remember your medication, but choose not to take it for some other reason? How often do you forget your medication compared to choosing not to take it? When you miss it, do you always miss all of your medication or just some of them?"
Identify barriers	Different determinants of non-adherence require different interventions.	"What gets in the way of taking medication? What is the worst/hardest thing about (having to take) medication?"

ing for their prescriptions and travel to hospital; when money is limited, these can be very real barriers to adherence for which support is available. Within our service, we adopt a multidisciplinary approach to identifying and managing adherence, which begins with a stance of assumed non-adherence, and reinforcing disclosures as rates are known to be around 50%, we normalize that most young people will struggle to take all of their medication all of their time. Please see Table 77.2 for details of our approach for routinely assessing in this age group. From conducting a case note review of the more complex cases seen by our Clinical Psychologist and Specialist Social Worker, we found that a significant minority had entrenched relational difficulties and had experienced childhood abuse [70]. We hypothesized that non-adherence can be related to attachment difficulties and in some cases can require long-term specialist input to treat. Effective identification of non-adherence and the factors contributing to it are essential to ensure access to the appropriate services.

Self-Management

As described earlier, adolescence and young adulthood for young people with liver conditions can be associated with poor health outcomes related to non-adherence and graft loss. Self-management relies on the engagement of individuals in order to manage their health effectively, in a pediatric setting implying support from the parents/carers. From a behavioral perspective, one of the simplest explanations for difficulties during transition is that some young people are just not yet good at managing their own healthcare [71]. Annunziato et al. demonstrated that in a cohort of young adult pediatric liver transplant recipients, self-management skills appeared to develop with age with lower scores for those transplanted before the age of 10 years compared to older age at liver transplant. Further work from the same authors raised some concerns that young adults post-liver transplant reporting greater self- management were being less adherent to treatment, and this impacted on their medical condition as it was

found to be associated with rejection. They concluded that universal promotion of self-management in young adult patients was inadvisable and that acquiring self-management skills should be viewed as a gradual process. Input from a multidisciplinary team lasting well into the mid-twenties was recommended [72–74].

With the delayed timing of role transitions in today's society, such as completion of education, marriage, and parenthood, many of our young people may continue to rely on parental support well into the period of "young adulthood." This may be especially true in our population, considering almost 50% of children post-liver transplantation require special educational support [47]. Our young patients may be more likely to struggle with the development of the appropriate skills to manage their condition during adolescence and may continue to rely on carer support. Indeed, most young people with childhood liver disease have a long history with the pediatric care providers, with many relationships starting in infancy. Transition of healthcare to adult services can therefore be just as challenging for carers themselves, who have their own relationships with clinicians and healthcare providers. In practice, many patients may physically transfer from receiving services at pediatric to adult-oriented facilities before they manage the requirements of their particular medical illness. It is important that self-management is viewed as a process rather than a one-off conversation, especially in patients with additional learning needs (found at an elevated rate in our patients). It is recommended that clinicians periodically assess developmentally appropriate skills of health management in order to understand patient education needs and their skill acquisition over time.

Also of relevance here is the evidence that almost 50% of children after liver transplantation and with chronic liver disease require specialist educational support hence one can expect this to impact on the development of the skills expected when moving on to adult services [62]. In addition, with evidence that adolescent development is continuing into the mid-twenties, expectations that 16- and 18-year-olds might be capable of managing their condition independently could be unrealistic (Lancet adolescence). We recently explored the self-management skills and adherence patterns in a cohort of 156 patients attending our multidisciplinary young adult clinic. Results: There was a trend toward increased mastery of self-management skills over time, with those ≥19 years reporting being more confident in behaviors related to arranging appointments and organizing medications compared to those ≤18 years.

Non-adherence is thought to be related to both the adolescent stage of development [6] and the process of transitioning into adult services at this risky period [67]. It is therefore crucial for every center to carefully consider how to transition their young people. This is reviewed below.

Transition from Pediatric to Adult-Centered Health Services

The adolescent health society defined transition in 1993 as a "Purposeful, planned process that addresses the medical, psychosocial and educational/vocational needs of adolescents and young adults with chronic physical and medical conditions from child-centered to adult-orientated healthcare systems."

In 2002 American Academy of Pediatrics published the following consensus: "The goal of transition in health care for young adults with special health care needs is to maximise lifelong functioning and potential through the provision of high-quality developmentally appropriate health care services that continue uninterrupted as the individual moves from adolescence to adulthood" [75]. Several reports, mainly in the transplant setting, have since been published, with worse outcomes for patients transplanted during adolescence and a decrease in 12-month mortality in renal transplant recipients as patients age from 20 to 30 years. This supports the concept that maturation and complete development occur after the age of 18 years [2]. It seems that to date the development of dedicated programs to optimize transition from pediatric to adult-centered care has mainly been driven by pediatric specialists, with currently no consensus as to how to implement or measure this or even define what a successful outcome is. A national survey of adult transplant hepatologists on transitional care after liver transplantation in the USA in 2015 provided interesting information on the perception of adult healthcare providers [76]. We subsequently carried out a similar survey in the UK, and the results are summarized in Table 77.3 [3]. Thirty-two percent of respondents did not have a transition strategy at their center and only 16% had a formal transition program. Not having ade-

Table 77.3 Comparison of survey of transition service in the USA and UK

Comparison of USA and UK Survey results	USA (%)	UK (%)
Formal transition programme	16	61
No transition strategy	32	22
Characteristics of YP attending clinic appointment		
Have adequate knowledge about their condition	70	62
Arrived to the appointment with parent/guardian	66	76
Barriers to transition		
Inadequate communication with paediatric provider	61	11
Patient/family dependence on paediatric provider	46	67
Poor adherence	72	56
Patients lack the capability to discuss the impact of their condition independently without the help of their parent/guardian	54	28
Parents/guardians manage their child's condition without engaging their child	49	44

quate knowledge about their condition was found to be present in a third of the patients. The majority of adult transplant hepatologists were confident with their own skills to manage young people but were concerned about the lack of ability of the young people to independently manage their condition and their poor adherence to treatment with similar observations in the UK. Both in the USA and UK, concerns were raised about dependence of families on the pediatric provider and their interference with the patient's management as a barrier to transition and were concerned as well as the prevalence of non-adherence to treatment.

The care for young people with liver disease should focus on providing appropriate care for young people with liver disease irrespective of whether they are looked after in pediatric or adult services.

Different models of transition programs have been described and will need to be developed depending on the setup and needs of the individual centers. Pediatric teams should focus on developing strategies to overcome barriers to an adequate transition including learning difficulties, social factors, patients in care, and patients with mental health problems and aim for an integrative process. With regard to congenital and rare conditions typically presenting in childhood, where adult teams might be less familiar in managing these conditions, management in specialized centers with pediatric expertise is recommended.

What about the success of adequate young people care? Experience in the renal transplant setting demonstrated that the introduction of an integrated pediatric/young adult joint transition clinic and care pathway improved outcome over a 4-year period, with no episodes of late acute rejection or graft loss compared to 35% graft loss in a group of patients who did not benefit from this service [35]. In our UK survey, we found that those centers with formal transition programs perceived young people to have better knowledge of their condition, have better adherence, and rely less on the pediatric providers (Table 77.4) [3]. It is relevant to include parents and carers in the process, to give them realistic expectations of adult healthcare services and help them to transition from care provider to a more supportive role for the young person. This entails nourishing the development of self-management skills which are essential to navigate within an adult healthcare setting. [77]. In this respect, it is important to use transition readiness tools to define a patient's individual needs and for a multi-professional team to address these.

A recent small pilot study in a group of 20 liver transplant recipients whose care was coordinated by a transition coordinator showed, compared to a historic group of 14 patients, improved adherence to treatment during the year before transfer to adult services. After transfer, tacrolimus standard deviation scores (SD) remained stable in the group supported by the transition coordinator compared to the historic group where the tacrolimus SD increased, suggesting poorer adherence [78].

Table 77.4 Comparison between centers with and without transition services

Comparison between centres with and without transition service	Transition service *N* = 9 (%)	No transition service *N* = 9 (%)
YP has adequate knowledge about their condition	76	50
Poor adherence	44	67
Patient/family dependence on paediatric provider	56	78

Ideally the timing of the transition process should be flexible and aimed at the patient's needs and readiness; however in practice, lack of age-appropriate inpatient facilities or pediatric and adult setting being on different sites often means that patients over 18 years cannot be admitted to pediatric inpatient facilities, raising the importance of starting the transition process early enough. This is particularly relevant in patients with special healthcare needs where the transition process becomes more complicated as the patient might not be able to advocate for their care, consent for procedures, and manage an inpatient stay on an adult ward independently. In these cases, the multiprofessional team should ensure a well-documented care pathway is discussed prior to transition to adult services [79, 80].

Summary and Conclusions

- With advances in medicine, more patients with liver conditions are growing into adulthood.
- Adolescence is a period of biological, psychological, and social changes, and the impact of a chronic condition on this process can be significant.
- Outcome data suggest that young people are a unique and vulnerable cohort who deserves special attention by health professionals, focusing on better outcome and survival.
- Growth failure and pubertal delay are prevalent, and sexual health advice should be offered standard during the consultation with the young person.
- Psychological aspects of growing up with liver disease are increasingly being recognized and identified and require management by specialized healthcare professionals.
- Non-adherence to all aspects of care is common, multifactorial, and often underestimated however impacts on outcome and survival. A non-judgmental approach aimed at identifying barriers to adherence and developing an individualized strategy is recommended.

- Successful transition programs have shown improvement in outcome and quality of life and should be developed according to the facilities of the individual center and focus on self-management, keeping in mind the special needs patients might have.

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New Horizons in Paediatric Hepatology: A Glimpse of the Future

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Emer Fitzpatrick and Anil Dhawan

Introduction

Over the last 30 years, considerable advances have been made in the field of paediatric hepatology as a distinct subspecialty. Paediatric hepatology is now poised on the brink of a hugely exciting era with the potential of advances in technology particularly in genetics, bioengineering and small molecules bringing real advantages to diagnostics and therapeutics. In addition, there is a renewed focus not just on survival and medical outcomes but on improving the quality of life for patients and minimising the side effects of each treatment.

As a speciality of rare diseases in general, much of the research and development to date comes adapted from adult practice. In the mid-twenty-first century however, with focus on genetics and metabolic disease, paediatric hepatology research is set to take the lead as regards innovative practice.

Genes, cells and molecules are cross-cutting themes across diagnostics, the better understanding of pathophysiology and thus therapeutic options. In addition, there is a renewed emphasis on the importance of psychosocial outcomes and quality of life. New challenges face us in the twenty-first century, particularly the massive increase in the prevalence of obesity across both the adult and paediatric population. This has consequences in terms of both lifestyle-related liver disease and the decline in healthy livers suitable for transplantation due to the frequent occurrence of steatosis.

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Genetics

Diagnostics

Our understanding of single gene and polygenetic/multifactorial disorders has undergone major advances over the last number of years and continues to do so. Many of the rare disorders described in paediatric hepatology have found explanation in genetic alteration thanks to collaboration of paediatric hepatologists and geneticists internationally. The major technological advances of next-generation sequencing, whether in whole genome analysis or targeted studies, have allowed analysis and elucidation of huge amounts of data. Over the last decades, the genetics of Wilson disease [1, 2]; Alagille disease [3, 4]; arthrogryposis, renal dysplasia and cholestasis (ARC); microvillus inclusion disorder; FIC1 disease; BSEP deficiency; MDR3 deficiency; TJP2 disease; and DCDC2 have been described [5–8]. In addition to this, the genetic basis of many metabolic diseases affecting the liver such as glycogen storage disease and mitochondrial depletion disorders [9, 10] has been discovered leading to more rapid and less invasive diagnostic measures. The previous 'black box' of conditions leading to acute liver failure has in part been opened with the discovery of genetic mutations such as LARS, NBAS, SCYL1 and EIF2SK3 [11, 12], and the panel of such mutations is continually expanding.

Gene 'chips' now exist for the diagnosis of cholestatic conditions in neonates, immunological and haematological disorders relevant to hepatology, acute liver failure and certain metabolic diseases. These 'chips' can detect the presence of mutations consistent with liver diseases and as they can usually be processed rapidly may be more useful than biochemical or other means of diagnosis and in some cases, may reduce the need for liver biopsy.

The interaction of genetic susceptibility and the environment has also come to the fore with an understanding of genetic variation in man due to epigenetics. Single-nucleotide polymorphisms can explain variation in susceptibility to disease processes, for example, PNPLA3 in NAFLD [13], auto-

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immune liver disease [14] but also in hepatocellular carcinoma and response to treatment in hepatitis C. Next on the agenda are determining the genetic modifiers for multifactorial conditions – for example, biliary atresia and idiopathic acute liver failure. Cystic fibrosis-associated liver disease and liver disease in those with alpha 1 antitrypsin deficiency will also benefit from this approach.

Gene Therapy

Genetic modification has still promise as a therapy. Liver-based metabolic disease has the particular advantage of the liver as a target organ for gene therapy. Preclinical studies and clinical trials to date have yet to overcome safety concerns.

The delivery of gene therapy involves use of a vector at present which may be viral or nonviral. This in addition to the major issue of introducing genomic instability is the major safety concerns limiting the current clinical applicability of the therapy.

Clinical trials for gene therapy in Crigler-Najjar syndrome and urea cycle defects have commenced, and those for organic acidemias (methylmalonic acidemia and propionic acidemia) are in development. Wilson disease is another potential target as are several others of the monogenic liver diseases of childhood.

Other approaches to genetic therapy targeting liver disease include using interfering RNA. Though this research has largely been abandoned by big companies, smaller biotech companies have reported some success including the use of the technique to treat transthyretin-mediated amyloidosis, a liver-centric disease [15]. Small interfering RNAs bound to the RNA-induced silencing complex mediate cleavage of target messenger RNA. This has been exploited by developing tools to enable delivery to target organ in stable formulation, for example, lipid nanoparticles which are stable and robust. When delivered intravenously, they home to the liver.

MicroRNAs (miRNAs) are small noncoding RNAs, 18–24 nucleotides in length, that regulate gene expression by binding to mRNAs to interfere with the process of translation. They are regulated during or after transcription, and SNPSs in miRNA genes may also modify their activity and function. Approximately 1400 mammalian miRNAs are known and each can influence 100 s of gene transcripts. These molecules are involved in all biological processes in all cell types. In the liver, there has been much interest in the role of miRNA in glucose and lipid metabolism, inflammation, apoptosis and cell death, cell cycle and proliferation and fibrosis. miRNA have been investigated as both biomarkers of disease processes in the liver and as potential therapeutic targets by both inhibition and overexpression.

Regenerative Medicine and Cell Therapy of Liver Disease

Liver transplantation is undoubtedly the standard of care for end-stage liver disease and many liver-based metabolic conditions and developments in surgical technique, peritransplant care, and immunosuppressive medication means that outcomes are improving. However the unfortunate fact is that organs are in short supply and the growing tendency towards obesity in the general population means that fewer and fewer non-steatotic, healthy livers are available through donation and are particularly unsuitable for split organ transplantation (which is the main source of transplanted livers in children). Thus, deaths inevitably occur while awaiting a suitable organ. Hepatocyte transplantation is becoming more established as an alternative to orthotopic or auxiliary liver transplantation in the management of liver-based metabolic conditions and in acute liver failure [16–22]. As with auxiliary transplantation, the native liver remains in place; in the case of acute liver failure, this allows the possibility of native liver regeneration over time; in metabolic liver disease, the native liver serves as a back-up in case of graft failure and remains available for the future possibility of gene therapy. The procedure is significantly less invasive than organ transplantation and previously isolated and cryopreserved cells are available on demand.

Hepatocytes are derived from organs that would otherwise be rejected for transplantation. In general, these often compromised livers may be steatotic, from non-heart-beating donors [23] or unused segments [24]. Innovative methods to improve cell quality from these poor grafts and cryopreservation methods have been a focus of research. Challenges also remain in the effective engraftment of hepatocytes into the host liver including optimal immunosuppression and loss of function after 6–9 months.

In acute liver failure, hepatocyte transplantation may act as a bridge to recovery and regeneration of the injured native liver or alternatively to orthotopic liver transplantation once an organ becomes available. The procedure may also be used in patients who are not candidates for organ transplantation. A major advantage of hepatocyte transplantation is the immediate availability of cryopreserved cells. Sufficient cell mass (approximately 10–15% of liver cell mass) is needed to provide enough function to sustain metabolic function [25]. In chronic liver disease, or acute on chronic dysfunction, the aim of hepatocyte transplantation is both to replace function and to allow the cells engraft and repopulate the liver. As the liver architecture is disrupted in chronically damaged liver, there are difficulties with engraftment however. Attempts at improving engraftment include temporary embolisation of the portal vein [26], liver irradiation [27] and the administration of growth factors [28]. The single most important obstacle to the

widespread application of hepatocyte transplantation is the limited availability of hepatocytes. This has encouraged investigation into alternative sources of cell including stem cells, immortalised cells and xenotransplantation each with its own challenges. Stem or progenitor cells have many advantages as an alternative source of cell for hepatocyte transplantation. They are readily available and may be effectively expanded in vitro or in vivo. Autologous cells (induced pluripotent cells) eliminate the need for immunosuppression and, however, may need to undergo corrective genetic manipulation prior to use to correct the underlying defect [29–32].

Proof of true functionality of these cells has been less frequent. It is not clear as yet whether transdifferentiation or fusion is responsible for change in phenotype of the cells. The tumorigenic potential of modifications to the genome needs to be considered.

Mesenchymal Stromal Cells

Mesenchymal stromal cells have also been shown to have promise in the cell therapy of liver disease [33]. These are multipotent, adherently growing cells which provide support for haematopoietic cells within the bone marrow [34]. They are a readily available source of stem cell also isolated from umbilical cord blood and matrix, placental tissue and adipose tissue [35–37]. Mesenchymal stem cells (MSCs) have beneficial effects in animal models of acute liver failure. They can transdifferentiate into hepatocyte-like cells both in vitro and in vivo [38, 39]. MSCs play a major role in tissue repair both through localised immune-suppressive effects and through the release of soluble trophic factors to affect neighbouring cells, properties which make them excellent candidates for improving the survival of transplanted cells [40]. Improved survival has been shown in animal models of liver disease and in pilot clinical studies of liver failure [41–43]. It is not clear, however, whether it is the transdifferentiation of MSCs into hepatocyte-like cells or an anti-inflammatory, anti-apoptotic effect induced by the MSCs results in the desired outcome. Soluble factors such as growth factors, cytokines, extracellular matrix glycoproteins and other small molecules produced by MSCs likely mediate these effects in acute liver failure rather than transdifferentiation [40]. This anti-apoptotic, pro-regenerative effect of MSCs has also been seen in the setting of myocardial infarction [44] and stroke [45]. In addition to the beneficial effects on liver inflammation and regeneration, other studies have recognised the supportive effects of bone marrow-derived cells in co-culture with hepatocytes [46]. Co-encapsulated hepatocytes and bone marrow stem cells resulted in prolonged maintenance of function in the co-encapsulated group in vitro and in vivo in a rodent model of acute liver failure [47–49]. Thus not only are MSCs are anticipated to improve the viability and functionality of co-transplanted hepatocytes. In addition, transplanted mesenchymal stem cells may also be a source of anti-inflammatory/anti-apoptotic mediators in the setting of the failing liver and the subsequent cytokine storm. MSCs are already used clinically in the treatment of graft versus host disease and have safety and logistical advantages over embryonic stem cells and adult-derived hepatocyte-like stem cells. A recent report of alginate encapsulated primary human hepatocytes together with mesenchymal stromal cells infused into the peritoneal cavity of children with acute liver failure demonstrated encouraging results [50]. A clinical trial is currently set up to explore the safety and efficacy of this approach further.

Induced Pluripotent Stem Cells

The description by Takahashi and Yamanaka [51, 52] of the induction of somatic cells back to their pluripotent state by overexpressing certain transcription factors and the subsequent differentiation of the cells along alternative lineages has opened massive possibilities for cell therapy in liver disease. The differentiation of autologous dermal cells into hepatocytes following induction of pluripotency may have significant advantages, avoiding the need for immunosuppression in cell transplantation and providing an accurate model of disease to investigate pathophysiological mechanisms and test therapies. Of course, the transplantation of autologous cells in a disease state would require the need for genetic manipulation to correct that defect in the first instance. Thus potential genomic instability may be a problem; however, new techniques are emerging which may minimise this risk and allow genomic stability. One such approach is using a piggyBac technique which enables removal of transgenes without residual sequences. This technique was used to good effect in a study by Yusa et al. which differentiated iPS cells from dermal cells of a patient with alpha 1 antitrypsin deficiency, corrected the genetic abnormality and demonstrated the function of the hepatocyte-like cells when transplanted into a mouse [53]. iPS-derived hepatocyte cells have also been used in the successful formation of ‘liver buds’ together with endothelial cells from umbilical cord and mesenchymal stromal cells. When transplanted into a mouse, this human liver bud rapidly vascularised and demonstrated liver-specific function [54].

Immortalisation

In order to establish a sustainable source of cells for transplantation, immortalisation of hepatocytes has also been described. Clonal cell lines of human and rat hepatocytes

have been transduced with retroviral vector expressing the immortalising simian virus 40 large T antigen gene [55]. Despite control mechanisms for switching immortalisation on and off, concerns regarding safety remain. The use of these cells in an extracorporeal device remains attractive.

Thus, the potential of cell therapy for liver disease is promising, though cell source is an issue and the emergence of mesenchymal stromal cells and iPS cells into the clinical field is particularly relevant and may provide an alternative source for successful translation. Paediatric liver disease, especially liver-based metabolic disease, remains a priority for this approach.

Small Molecules and Chaperones

A reduction in protein stability is often a common outcome of monogenic disease, for example, alpha 1 antitrypsin deficiency or cystic fibrosis; thus, focus on the stabilisation of such proteins and provision of an increase in intracellular function are the remit of pharmacological chaperones and other small molecules.

The work of Lomas group and others has focused on the use of small molecules to block the abnormal polymerisation of alpha 1 antitrypsin in the Z state [56]. At present, however, this work is proof of principle and not yet at translation point, though in the future may offer real therapeutic promise. A paediatric group in Pittsburgh investigated the use of carbamazepine as an autophagy enhancer to clear the intracellular load of abnormally formed alpha 1 antitrypsin in the disease state and reduce liver fibrosis in mice [57], using a well-established pharmacological substance as a molecular manipulator. Similarly lysosomal storage disease and phenylketonuria have also been a focus for the development of protein chaperones [58, 59].

Targeting other nuclear receptors with pharmacological agents is a focus for polygenic or multifactorial disease such as NAFLD and primary biliary cirrhosis. FXR is a nuclear receptor that binds extensively to genomic DNA. FXR undergoes conformational change and alters gene expression following binding by bile acids or other pharmacological compounds. This results in the regulation of bile acid levels in the liver and in addition glucose and lipid homeostasis. Thus modulation of FXR binding and function using a semi-synthetic bile acid may be a useful approach to therapy of NASH [60] and certain cholestatic liver diseases for which there are early clinical studies ongoing at present [61]. The apical sodium-dependent bile acid transporter (ASBT) is also a target for inhibitors in clinical trial for the treatment of severe pruritus in cholestatic liver conditions [62].

Liver Transplantation

Now that survival following liver transplant is greater than 90%, the focus has become more about improving long-term quality of life and minimising side effects of immunosuppressive medications. Though short and medium survival is very high, longer-term graft loss and renal dysfunction, post-transplant lymphoproliferative disease and other side effects of immunosuppressive medication are the major issues that must be addressed. A better understanding of late graft loss and the agents involved should contribute to achieving tolerance for a lucky few. True tolerance is a rare occurrence in paediatrics, but individual immunological 'signature' detected with new technology may identify those who are candidates. These areas will be of interest in both paediatric and adult settings but the anticipated longevity of children make this even more relevant for paediatrics.

The Microbiome and Liver Disease

The microbiome and its role in many paediatric liver disease are a major area of interest currently, and should the ongoing studies prove the concept, this is set to be a major area of development over the next 5 years. The role of microbiota in metabolism and in immunity/inflammation is becoming clearer. The relationship between the gut microbiome, bile acids and liver disease is the subject of current study, and exciting new therapies may arise from this understanding. The hypothesis is that a decrease in delivery of bile acids to the small intestine as may occur in cirrhosis or in cholestasis results in dysbiosis which via metabolism of pathogenic bacteria, lipopolysaccharide (cell wall) and translocation exacerbates liver inflammation and thus promotes fibrosis. Hepatic encephalopathy has also been associated with dysbiosis. Modification of this dysbiosis using pre- or probiotics may be effective as therapy and is under investigation in NAFLD in particular in addition to the modification of hepatic encephalopathy in cirrhosis. Vancomycin is also under trial in both sclerosing cholangitis and biliary atresia. In addition to bacteria and antibiotics, TLR54 antagonists may have a role as an anti-fibrotic therapy. Microbial genomics and computational biology has a significant role to play in understanding the gut-liver axis. Considering 99% of genes in the human body are bacterial, there is a huge amount of data to collate and analyse in understanding the contribution of these genes to diversity.

Non-alcoholic Liver Disease

NAFLD in children is now a major public health issue with serious concerns about the burden that this will place on the adult service. It is not known what proportion of children

with NAFLD will go on to develop end-stage liver disease and/or hepatocellular carcinoma in early adulthood, but with early-onset progressive disease, there is a risk that end-stage liver disease secondary to NAFLD will overwhelm the transplant capacity. Though multiple clinical trials are underway investigating potentially disease-modifying medications, the mainstay of management in children is lifestyle change with healthy eating and exercise. Lifestyle and environmental factors are not the only culprits however, and genetic susceptibility has been demonstrated by genome-wide studies. In utero and early infancy exposures are also thought to prime an individual to metabolic risk. Well-designed randomised controlled trials (such as the TONIC trial in children with NAFLD) are needed to evaluate the role of other drugs such as anti-fibrotics, antioxidants and drugs which modify the metabolism and other newer therapies for this condition, but likely it is only a public health approach starting prior to pregnancy that will result in significantly improved outcomes.

Noninvasive Biomarkers of Disease

Though the role of liver biopsy in paediatric hepatology remains important, noninvasive methods of detecting and assessing progression of disease are becoming more accessible. Algorithms of simple biochemical and clinical markers such as paediatric fatty liver index for NAFLD and APRI for detection of varices in chronic liver disease have demonstrated their utility. Ultrasound-based techniques such as transient elastography and shear wave elastography are now well established as surrogates for liver biopsy to determine severity of fibrosis. Contrast ultrasound can distinguish different types of tumour and assist in assessing liver trauma.

Recent MRI protocols can evaluate not only fibrosis (MR elastography) but also inflammation, fat and iron with multiparametric imaging.

Quality of Life

Finally, there is a need to acknowledge the ethical framework around management of children with liver disease. With a renewed focus on best quality of life, liver transplantation is now no longer a lifesaving measure alone. The timing around transplantation is a critical issue and no longer reserved for those with decompensated end-stage liver disease. In certain scenarios, the availability of a donor will have a major influence on this decision – once a child reaches a certain weight, a left lateral segment is insufficient and a right lobe will be required which obviously conveys further risk to living donor. School achievement and the presence of minimal encephalopathy are likely to become even more important in decision-making. There is a need to develop tools to accurately assess these criteria in children so as to time transplant in order to give them the best chance of educational attainment. Chronic pruritus and in the case of liver-based metabolic disease, frequent (even mild) decompensations leading to school absence are becoming accepted criteria for transplantation. In an era where organs are scarce, the complexity of meeting demand with supply is an ever-growing challenge.

Conclusion

This is an exciting and dynamic era in paediatric hepatology. Dramatic advances in genetic analysis, computational biology, understanding the microbiome and the gut-liver axis will lead to decoding many of the elusive concepts in paediatric hepatology. Regenerative medicine and cell therapies together with small molecules as chaperones have real potential for development and clinical application over the next decade. Finally, the recognition that quality of life in children with chronic liver disease and those post-transplant is often compromised will lead to a renewed focus on minimising side effects of medications and promoting early and effective intervention.

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